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EDITED BY

SIMON FLEXNER, M.D.

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THE AGGLUTINATION REACTIONS OF THE MORGAN BACILLUS NO. 1.

By I. J. KLIGLER, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 31, 1919.)

In the course of an investigation on the problem of soil pollution, bacilli having the cultural properties of the Morgan bacillus No. 1 were isolated from three polluted wells. The epidemiological significance of these findings is apparent provided that this organism really bears the relation to infectious diarrhea claimed for it by its discoverer. It was not feasible to enter into the question of etiology, but it has seemed desirable to compare the strains isolated from the polluted wells with some obtained from human sources. The resulting observations are of interest because they throw some light on the nature of this bacillus, which has been the subject of a great deal of discussion.

This organism was first described by Morgan in 1906. It belongs to the colon-typhoid group, but is peculiar in that it ferments only the hexoses with the production of gas. In the first epidemic of infectious diarrhea in which Morgan observed this organism he succeeded in isolating it from the stools of 40 out of 112 patients. In subsequent epidemics in England Morgan and his coworkers as well as Ross, Alexander, Lewis, and others, paid special attention to this type of bacillus. The reported findings are greatly at variance. Morgan isolated it in 54 per cent of the cases in 1905, 56 per cent in 1906, 16 per cent in 1907, and 53 per cent in 1908. Ross, Orr, and Alexander found this bacillus together with other non-lactose-fermenting bacteria, but claim that no one type was conspicuous. Alexander reports that during 1911 and 1912 the Morgan bacillus No. 1 was found in the stools of 4 per cent of normal and in 13.4 per cent of sick children examined. Lewis, on the other hand, in 1910 failed to isolate the organism from the stools of normal children, whereas he found it in 30 per cent of cases suffering from acute diarrhea. In 1911 he isolated the bacillus from 17 per cent of normal children and 70 per cent of the cases of diarrhea.

This peculiar organism has also been cultivated from sources other than human. Morgan and Ledingham isolated it from one cow out of eighteen examined; Lewis obtained it from five out of twenty mice. Cole isolated two strains from the intestinal contents of roaches.

The pathogenicity of the strains is variable. Both Morgan and Lewis proved that some strains were fatal for rats, mice, and rabbits when given *per os*. Neither Morgan nor Lewis was able to demonstrate the presence of specific antibodies in the sera of children from whom the bacilli were isolated.

The agglutination reactions of a large number of strains against different sera were reported by Lewis in 1912. He tested 242 strains from 167 cases, including those isolated from normal individuals, mice, cow, etc. against 16 sera. He found three main groups containing respectively 48, 17, and 8 per cent of the strains. There was no correlation between agglutination group and source. 11.5 per cent of the strains did not agglutinate with any of the sera. Lewis did not carry out any absorption tests.

It is evident from this brief review that the etiologic significance of the Morgan bacillus is by no means established. The work of Flexner and his coworkers, TenBroeck and Norbury, and Smillie leaves little room for doubt that in this country infectious diarrhea of infants is mainly caused by the dysentery bacilli. There remains, however, the possibility that the Morgan bacillus does play a minor part, since reports of its isolation frequently appear in the literature.

EXPERIMENTAL.

Strains Studied.—The total number of strains tested was small but representative. In addition to the three strains isolated from polluted water there were fourteen others, obtained from a variety of sources. Three came from the Lister Institute, England; two were from the Institute of Public Health, Canada, and were originally sent there by Captain Fidlar from Mesopotamia. Three cultures were isolated in this country from stools of infants suffering from acute diarrhea. The others were either stock strains of unknown origin or recently isolated from other sources. Table I gives the number, source, and, when known, the year of isolation of the cultures used.

Cultural Characters.—Morphologically and culturally all the strains were identical and corresponded with the original description given by Morgan. They were all motile, Gram-negative bacilli, resembling the other members of the typhoid-dysentery group. They fermented the hexoses (dextrose, levulose, and galactose) with the production of acid and gas, but failed to ferment any of the other fermentable substances tested (mannite, maltose, lactose, saccharose, xylose,

arabinose, rhamnose, salicin, and dulcite). In semisolid agar containing glycerol and Andrade indicator they produced acid in the upper (aerobic) and not in the lower (anaerobic) part of the tube. All of them produced an abundance of indol in 24 to 48 hours in beef infusion broth. Gelatin was not liquefied. In their behavior toward the triphenylamine dyes they were more akin to the typhoid than to the dysentery bacilli.

TABLE I.

List of Cultures Used.

Strain No.	Source.
M38a	Lister Institute, England. Origin not given.
M45a	" " " " " "
M33	" " " " " "
M.J.	Institute of Public Health, Canada. Obtained from Captain Fidlar, Mesopotamia.
M.D.	Institute of Public Health, Canada. Obtained from Captain Fidlar, Mesopotamia.
139	Stock strain from the American Museum of Natural History, isolated from water.
T17	Isolated, 1917 from the stool of a normal individual.
57	" 1917 " polluted well water.
318	" 1917 " " " "
25	" 1918 " " " "
M.R.	" 1916 " the intestine of a roach (Cole).
M.RX	" 1916 " " " " " " "
586	" 1915 " " stool of an infant (diarrhea).
692	" 1915 " " " " " " "
M.K.	Received from Professor Kendall. Origin unknown.
M.T.	" " Dr. TenBroeck. Isolated Aug., 1914, from a case of infant diarrhea in Boston.
S2	Isolated, 1917, from polluted soil.

Pathogenicity.—The pathogenic property of some of the strains was tested by feeding them to white mice and rats. The method of feeding was as follows: Veal broth containing 10 gm. of fresh veal to 100 cc. of the broth was used. The inoculated tubes were incubated for 48 hours at 37°C., and 10 cc. of these cultures fed to each animal by soaking the bread fed to them. Some of the animals were fed 10 cc. of culture on 2 consecutive days. None of the mice or rats became ill, although the bacilli were abundant in the feces for

several days after the feeding. The bacilli usually disappeared from the feces within 5 to 10 days after the feeding. Strains 692 and 586, most recently isolated from cases of acute diarrhea, and Strains 57, 318, and 139, obtained from polluted water, were employed in these tests.

Agglutination Reactions.—Agglutination tests were made with nine sera. The sera were prepared by injecting saline suspensions of

TABLE II.

Agglutination Reactions of Morgan Bacilli in Different Sera.

Strain No.	Agglutination with Serum.								
	692	586	139	318	M. K.	T 17	33	45	38
M38a	—	—	—	—	—	1,280*	320	2,560	2,560
M45a	—	—	—	—	—	2,560	640	2,560	640
M33	—	—	—	—	—	1,280	1,280	1,280	2,560
M.J.	1,280	—	80	—	—	—	—	—	—
M.D.	—	—	—	—	2,560	2,560	—	40	80
139	—	—	1,280	—	—	—	—	—	—
T17	—	—	—	—	320	2,560	—	2,560	640
57	—	40	—	80	—	—	40	—	—
318	—	—	—	2,560	—	—	—	—	—
25	—	—	1,280	—	—	—	—	—	—
M.R.	—	—	40	—	80	1,280	—	—	—
M.RX	—	—	—	—	—	—	—	—	160
692	1,280	—	—	—	—	—	—	—	—
586	—	1,280	—	—	—	—	—	—	80
M.K.	—	—	—	—	2,560	2,560	—	640	—
M.T.							160	—	—
S2	—	1,280	—	—	—	—	—	—	—

* The numbers indicate the highest dilution at which agglutination occurred.

24 hour growths on agar slants. Five injections at 3 day intervals were usually given. The results are summarized in Table II. The numbers in the table indicate the highest dilution in which agglutination occurred. It is evident at a glance that the strains are highly diversified antigenically. The seventeen strains fall into six distinct groups.

Absorption Tests.—The heterogeneity of these strains is more strikingly shown by the absorption tests. Cultures T17 and M.K., for

example, cross-agglutinated in high dilutions, but their respective sera behaved differently towards the other strains. Absorption tests

TABLE III.

Absorption of Agglutinins from Sera Produced against Various Strains of Morgan Bacilli.

No. of agglutinating culture.	Serum 38 absorbed with.														
	Culture 38.					Culture 33.					Culture 45.				
	80	160	320	640	1,280	80	160	320	640	1,280	80	160	320	640	1,280
38	++	+	−	−	−	++	++	++	+	+	++	++	++	++	+
33	+	±	−	−	−	++	++	+	−	−	++	++	++	+	−
45	−	−	−	−	−	+	±	−	−	−	++	++	+	−	−
T17	−	−	−	−	−	+	±	−	−	−	++	++	±	−	−
	Serum 45 absorbed with.														
	Culture 45.					Culture T17.					Culture 38.				
38	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
33	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
45	+	−	−	−	−	±	−	−	−	−	++	++	++	+	−
T17	+	−	−	−	−	±	−	−	−	−	++	++	++	+	−
	Serum 33 absorbed with.														
	Culture 33.					Culture 45.									
33	−	−	−	−	−	++	++	++	++	++					
45	−	−	−	−	−	−	−	−	−	−					
	Serum T17 absorbed with.														
	Culture T17.					Culture M. K.									
T17		−	−	−	−	++	++	++	++	++					
M.K.		−	−	−	−	−	−	−	−	−					
	Serum M. K. absorbed with.														
	Culture M. K.					Culture T17.									
T17	−	−	−	−	−	+	±	−	−	−					
M.K.	+	−	−	−	−	++	++	++	++	++					

showed that they were distinct types. Similarly, the sera produced against the English strains M38a, M45a, and M33, cross-agglutinated the heterologous strains in high dilution, but absorption tests

showed that the three organisms were antigenically different. The seventeen strains are thus separated into ten distinct antigenic types without any correlation as to source. The results of the absorption tests are given in Table III.

SUMMARY AND CONCLUSION.

The study of the cultural and agglutinating reactions of seventeen strains of Morgan bacilli is reported. The cultures were obtained from different sources. Culturally all the strains were identical. Antigenically they were highly diversified. The sera produced against some strains have marked agglutinating power for other strains, but absorption tests showed that the cross-agglutinations were often due to group agglutinins. While no conclusion can be drawn regarding the pathogenic significance of this bacillus, the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathological processes in man.

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EDEMA OF THE LUNGS AS A CAUSE OF DEATH.*

By M. C. WINTERNITZ, M.D., and R. A. LAMBERT, M.D.

(*From the Department of Pathology of Yale University School of Medicine, New Haven.*)

(Received for publication, April 3, 1919.)

Edema of the lung is a frequent terminal event in the course of many diseases, both acute and chronic, and in such cases is commonly interpreted as the immediate cause of death. It constitutes, as is well known, a striking feature in poisoning by most of the noxious gases in modern warfare. It is, therefore, not surprising that in the clinical and pathological reports of these fatal gas cases, death should be regularly attributed to pulmonary edema.

The question as to how edema of the lungs may bring about death has long been discussed. The view generally accepted is that the fluid in the pulmonary alveoli interferes mechanically with gaseous interchange and that when this interference with respiration passes a certain critical point, the patient dies of asphyxia; that is, he drowns in his own fluid. It is not our purpose in this paper to discuss the question as to how edema of the lungs may cause death, but rather the fundamental problem of whether the mere accumulation of fluid in the lung is of itself a serious matter.

During the past 2 years we have had the opportunity of studying the effects of practically all the commonly used war gases under laboratory conditions. About 3,000 dogs and an equal number of other animals have been exposed to gas and then observed clinically and at autopsy. Particular attention has been given to the pulmonary edema, which, as in man, is a fairly constant and, in many cases, a striking phenomenon of the gassed state. These observations, together with the results of some experiments upon what

* This work is an incidental result of more detailed studies in the pathology of war gases which were conducted under the auspices of the Bureau of Mines, the Chemical Warfare Service, and the Office of the Surgeon General, U. S. Army.

may be termed an artificial pulmonary edema produced by filling the lungs of a normal dog with an isotonic salt solution, have led us not only to question the importance of pulmonary edema *per se* as a cause of death, but to conclude that edema of the lungs in general is merely an indicator of some underlying disorder and is rarely, if ever, directly responsible for the death of the patient or animal. These observations are summarized in the following paragraphs.

Animals which die acutely from exposure to any of the gases of the respiratory irritant group, such as chlorine and phosgene, show at autopsy varying degrees of edema of the lungs. Although this is regularly well marked in certain species, dogs for example, there are wide individual variations. In other species, rats and guinea pigs for example, it may be a relatively inconspicuous feature in spite of the fact that these animals are particularly susceptible to the gas.

Likewise dogs which are killed before the action of the gas reaches its maximum effect show striking differences in the amount of fluid in the lungs, and these differences do not harmonize with the variations in the symptoms manifested by the animals. Furthermore, many dogs which pass successfully the critical 48 hour period and are classed as "recovered" often show, when killed, edema of the lungs of greater degree than other dogs of the same experiment which succumbed.

The increased concentration of the blood in the gassed animal, which some investigators regard as a reliable index of the condition of the animal and as a guide to therapy, does not correspond necessarily in any measure with the degree of pulmonary edema present.

The lungs of a normal animal may be filled with isotonic salt solution, thus producing an artificial edema comparable with that found in the gassed state.

Several of the propositions set forth require elaboration and discussion. They are considered in detail below.

Variations in the Degree of Pulmonary Edema in Gassed Animals.

The gross and microscopic picture of the lungs associated with acute gassing has been so thoroughly described in the papers published from this laboratory and elsewhere as to need no repetition

here.[1] As pointed out above, edema of the lungs in dogs is generally well marked, and with the associated congestion constitutes the most striking feature of the autopsy findings. However, among individual dogs dying in the acute stage the degree of edema has been found to vary within relatively wide limits. These differences are obvious to the naked eye, but in order to make satisfactory comparisons, free from the personal equation, we adopted the following method for measuring the degree of edema. This method, which is based on the comparative weights of the lung and empty heart, was suggested and used by Barcroft in the investigations carried on by the British Research Committee.

Method.—The lungs are weighed with the trachea attached, cut to uniform length, and clamped to prevent the escape of edema fluid. The heart is also trimmed uniformly and completely emptied. The normal ratio of the lung weight to the heart weight was obtained by taking an average of sixteen normal animals. This normal ratio was found to be 1:30. Barcroft's higher figure (1:50) is probably the result of a difference in the method of trimming the organs. In gassed dogs we have divided the lung weight by the heart weight, and this quotient by the normal ratio, 1:30. We have termed the resulting figure the "edema index." It represents the percentage increase in lung weight over the normal. This method has been objected to by Eyster[2] who insists that the dried weight method is much more reliable. There are two of these dried weight methods; one, used by some of the French investigators, in which a typical slice of the lung is weighed wet and then after drying, and the second, the method used by Eyster and his assistants,[2] in which the entire lung is dried, the proportion between the wet and the dried specimen indicating the degree of edema. The first method is obviously open to large errors. The second method has the serious drawback of making it impossible to study the lung grossly or microscopically. We have compared the latter method with our more

[1] Winternitz, M. C., and collaborators, Collected studies on the pathology of war gas poisoning, New Haven, 1919; Anatomical changes in the respiratory tract initiated by irritating gases, *Mil. Surg.*, 1919 (in press).

[2] Eyster, J. A. E., Report of the Chemical Warfare Medical Committee, Medical Research Commission (unpublished).

simple lung-heart ratio method in a series of six dogs, and have found that the two methods give results which are approximately the same (compare Columns 9 and 13 in Table I).

TABLE I.

Relation of the Edema of Lung and the Concentration of Blood in Gassed Animals.

Dog No. and breed.	Gas.	Concentration per liter.	Body weight.	Weight of empty heart.	Weight of wet lung.	Weight of dry lung.	Ratio of wet-dry lung.	Ratio of weight of lung-heart.	Red blood corpuscles before gas.	Red blood corpuscles before death.	Percentage increase of red blood corpuscles.	Edema index.
		mg.	*kg.*	*gm.*	*gm.*	*gm.*						
1. Hound.	Phosgene.	82	14.0	155	715			4.60	5,148,000	7,936,000	54	3.53
2. "	"	80	18.5	159	760			5.40	6,068,000	10,870,000	79	3.42
3. Mastiff.	"	75	26.0	170	915			5.40	8,400,000	10,808,000	28	4.15
4. Hound.	"	82	17.8	154	822			5.34	7,172,000	7,640,000	7	4.10
5. Collie.	"	99	17.8	177	570			3.22	6,192,000	11,260,000	82	2.48
6. Mongrel.	"	102	6.4	53	320	27.5	11.7	5.93	8,280,000	10,760,000	30	4.56
7. "	"	112	8.6	92	400	37.0	10.8	4.35	9,385,000	9,824,000	5	3.34
8. "	"	109	11.0	93	375	38.0	9.8	4.04	7,520,000	8,432,000	12	3.11
9. "	"	109	6.8	69	219	24.5	8.9	3.16	9,848,000	9,055,000		2.44
10. Setter.	Chloropicrin.	949	13.6	132	631	61.0	10.3	4.77	8,040,000	12,120,000	51	3.68
11. Mongrel.	Chloropicrin.	840	9.5	103	600	46.0	13.1	5.84	6,276,000	6,458,000	3	4.50
12. Bull.	Chloropicrin.	887	13.6	138	684	66.0	10.3	4.75	7,176,000	9,068,000	23	3.66

The dogs were killed 8 to 13 hours after exposure to phosgene or chloropicrin. Comparison of the figures in the last two columns (edema index and percentage increase in red blood cells) shows that no parallelism exists between the degree of pulmonary edema present and the blood concentration.

In using the lung-heart ratio method of estimating the amount of edema fluid present, we have found that the edema index in a series of dogs gassed under similar conditions varies within relatively wide limits. For example, among 50 dogs dying after exposure to phosgene the edema index ranged from 1.73 to 4.60, and in another series gassed with chloropicrin the extremes were 1.65 and 4.22.

In order to throw further light on the question of the significance of the degree of edema, the following experiment was done. Eight

dogs were gassed with phosgene (concentration 80 to 90 mg. per liter for 30 minutes). Four of the dogs died in from 10 to 15 hours. The remaining four were killed by chloroform. It was found that the average edema index of the four dogs that died was practically the same as that of the four that were killed. It was found also that many of the dogs which passed successfully through the critical 48 hour period and were then placed in the "recovered" group showed, if killed at this stage, a high edema index, often exceeding that of the dogs which had succumbed. It may be stated incidentally that these "recovered" dogs showed no symptoms other than occasional coughing and a slight sluggishness.

Still further evidence of the subsidiary part of edema as the cause of death after inhalation of irritating gases is found in the comparative effects of a gas such as phosgene on animals of different species.

A series of experiments was performed in which a number of different kinds of animals were exposed in the same chamber for 30 minutes to a concentration of 0.27 mg. per liter of phosgene. The time of survival varied as indicated in Table II.

TABLE II.

Species.	Time of survival.
Monkey	3 hrs., 30 min.
Guinea pig	4 " 30 "
Rat	5 "
Rabbit	11 " 30 min.
Mouse	Killed after 12 hrs.
Dog	" " 12 "
Goat	" " 12 "

The lesions produced in these animals by inhalation of phosgene are essentially alike. In the monkey and goat, for example, which represent the two extremes of susceptibility after exposure to the same concentration, lesions of the lung vary in degree but not in character. The species variation, evidenced by the length of the survival after gassing, in animals which have been killed or have died, may be expressed in part by the rate of development of the pulmonary edema. On the other hand, with some animals (monkey,

guinea pig), the first to succumb to a given concentration show less pulmonary edema than those that survive longer (dog, goat). This is evidence that the edema is in itself not the cause of death but simply one manifestation of a more important underlying change.

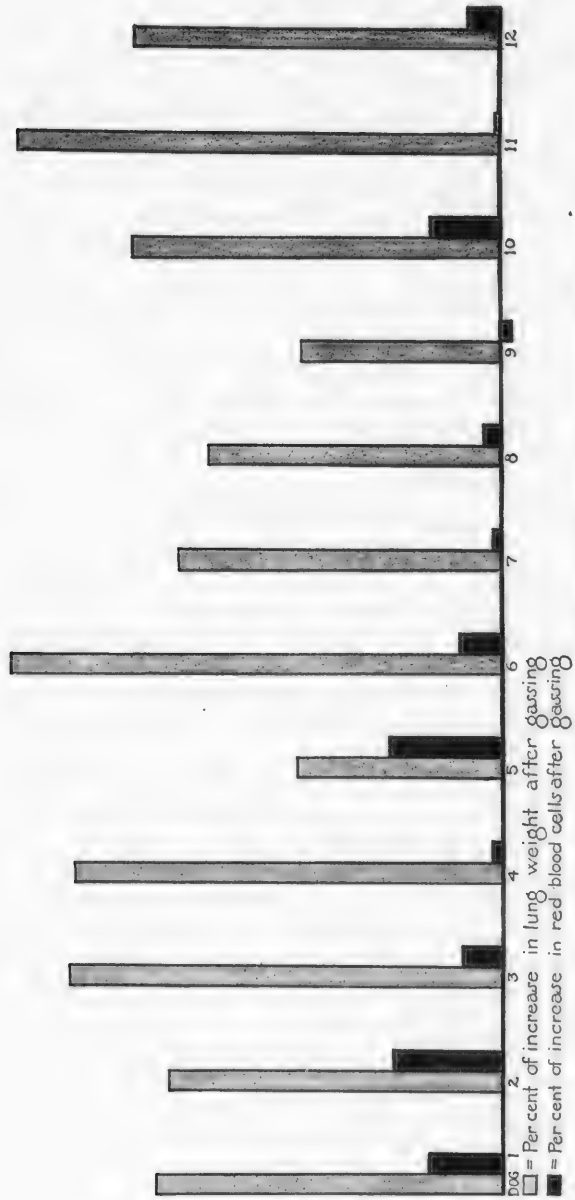
While the pulmonary edema develops more rapidly the more susceptible the species (Table II) the most susceptible show at death less edema than the more resistant ones. This is an indication of the importance of the time interval in the production of the edema.

Relation of the Concentration of Blood and Edema of the Lung after Inhalation of Gas.

Underhill[3] has found that dogs exposed to phosgene, chlorine, or chloropicrin show after a few hours (the time varies with different gases and with individual animals) a well marked increase in the concentration of the blood. Similar changes in the blood of gassed soldiers have been demonstrated repeatedly. The formed elements of the blood as well as the inorganic salts share in the change. Inorganic salts, however, do not follow the same course as the erythrocytes. The result is a marked increase in blood viscosity. Underhill and his assistants have used this blood change as an index of the condition of the gassed animals, and upon it have worked out a method of therapy, the essentials of which are bleeding and subsequent dilution of the residual blood with isotonic salt solution. In applying this method of treatment, which, it may be stated has definitely reduced the mortality among experimentally gassed dogs, it has been assumed that the concentration of the blood is due to the escape of blood serum into the lungs, and that, therefore, the increased viscosity of the blood may be taken as a rough index of the degree of pulmonary edema.

In order to determine whether or not these two phenomena, blood concentration and pulmonary edema, are directly related, the following experiment was carried out. Twelve dogs were gassed, nine with phosgene and three with chloropicrin, the duration of exposure and concentration being such as would be fatal to a majority of dogs exposed. The dogs were killed with chloroform about 10 hours after

[3] Report to be published in the near future.



TEXT-FIG. 1. Comparison of pulmonary edema and blood concentration after gassing.

the exposure to the gas; that is, as soon as the majority began to show serious symptoms. A red blood cell count was made before gassing and again just before the animal was chloroformed, since it has been shown that this is a reliable method for estimating the degree of blood concentration. The degree of pulmonary edema found was determined by the method described above. The figures for the increase in blood concentration and the edema index are recorded in Table I with other data. The results are also graphically shown in Text-fig. 1. It is seen that under the condition of these experiments no parallelism exists between the amount of fluid present in the lung and the degree of concentration of the peripheral blood. It is noteworthy that in one case in which there was a well marked edema of the lung, an actual reduction of blood concentration was found.[4] This experiment does not indicate that the loss of fluid from the blood may not have taken place by way of the lungs and the mouth, but, in our opinion, it does show conclusively that the change in the blood does not serve as an indicator of the amount of fluid present in the lung at any given moment. It suggests further that a therapy guided by the viscosity of the blood cannot be assumed to have any influence on the pulmonary edema, and that the beneficial results obtained by such therapy are probably in no way referable to a change in the fluid content of the lung, which of itself is of secondary importance, as will be emphasized in the following paragraph.

Artificial Edema of the Lungs. Pulmonary Irrigation.

Winternitz and Smith[5] have shown that the lung is much less susceptible to the introduction of fluid than has been generally supposed. Repeated experiments have demonstrated that the lungs can be entirely flooded through the bronchi with isotonic salt solution and that this process of irrigation can continue for at least 2 hours with the introduction of 6 liters of fluid without causing any evident

[4] There is a dilution of the blood, as shown by Underhill, which precedes its concentration with phosgene poisoning. These changes of blood concentration may vary somewhat in duration, etc., and explain the charted findings above.

[5] Winternitz, M. C., and Smith, G. H., Sir William Osler Anniversary Volume, New York, 1919.

harmful changes in bodily well-being or any subsequent serious microscopic lesions in the lung tissue. By means of the use of colored solutions it has been shown that the fluid introduced actually passes throughout the lung, bronchi, bronchioles, and alveoli and does not simply flow back through the trachea without entering the lung.

ETIOLOGY OF YELLOW FEVER.

I. Symptomatology and Pathological Findings of the Yellow Fever Prevalent in Guayaquil.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 31 to 34.

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In this paper it is proposed to describe the clinical features and pathological changes observed in yellow fever cases admitted to the Yellow Fever Hospital in Guayaquil.[1] It may be stated at the outset that in an analysis of 172 cases no clinical or pathological facts were brought to light which had not been described by previous students of yellow fever in Guayaquil[2] or elsewhere. The yellow fever cases occurring in Guayaquil present no special feature; the disease is classic in all its aspects. For the sake of clearness the general clinical features and the individual symptoms of the disease will be described separately.

[1] This hospital is under the direction of Dr. Wenceslao Pareja. Dr. Pareja not only pointed out many interesting clinical features but also performed autopsies for the members of the Yellow Fever Commission during their sojourn in Guayaquil. I am greatly indebted to Dr. Pareja for his cooperation in my investigations and to Dr. Charles Elliott of the Commission for permission to use some of his clinical notes made on about half the total number of cases coming under my observation during my stay. The Yellow Fever Commission of the International Health Board was composed of Dr. Arthur I. Kendall, Dr. Charles A. Elliott, and Mr. Herman Edward Redenbaugh of Northwestern University Medical School, Chicago; Dr. Mario Lebredo of Las Animas Hospital, Havana, Cuba; and Dr. Hideyo Noguchi of The Rockefeller Institute for Medical Research, New York.

[2] Strong, P. P., Tyzzer, E. E., Brues, C. T., Sellards, A. W., and Gastiaburu, J. C., Harvard School of Tropical Medicine, Report of the first expedition to South America, 1913, Cambridge, 1915, 180–200.

Clinical Features.

General Symptomatology.

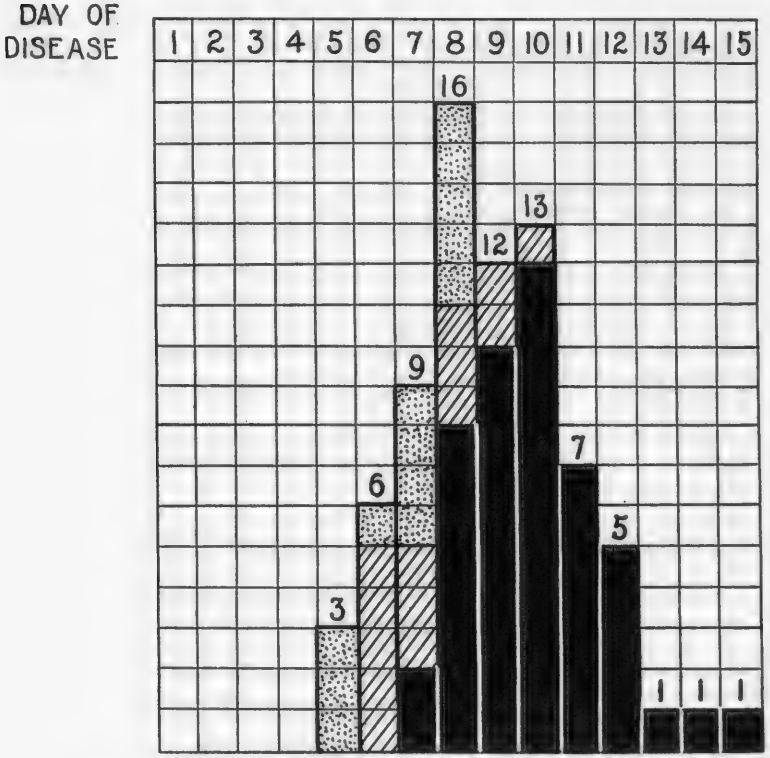
The incubation period varies from 3 to 6 days. It is difficult to determine the maximum. There may be prodromal symptoms for 1 or 2 days, but the onset is usually sudden, ushered in by fever, with or without chills. The patient feels gravely ill and in most cases goes to bed at once, with severe headache, pains in the loins, and anorexia; myalgia of the back, neck, and limbs is often intense, especially on pressure. Nausea and vomiting are frequently present. Insomnia and prostration follow. There is almost always tenderness in the epigastric region which gradually becomes aggravated as the disease progresses. The tongue has a heavy white coat with red tip and edge; later it may become brownish and dry. A peculiar, cadaveric-like odor emanates from the mouth. The gums are congested, swollen, and show a tendency to bleed on pressure. There is great thirst. The conjunctivæ are markedly suffused, becoming yellowish on the 2nd to 3rd day, sometimes with a few ecchymoses on the 6th to 7th day. The icterus of the conjunctivæ increases in the several days following and may persist 2 to 3 weeks in severe cases, although in milder ones it disappears in about 7 days. The black vomitus may occur on the 1st day or as late as several days after onset, or it may only be found in the stomach at autopsy. The skin is usually dry and icteric, and the patient suffers from intense epigastric pain. The gums may bleed profusely at this stage. The urine is reduced in volume; in many cases there is anuria for a day; the urine is dark, greenish, or brown, with abundant albumin and casts. Epistaxis occurs in many cases. Hiccoughs and other nervous symptoms (delirium, coma, convulsions) due to uremia and cholemia are frequent. Death may occur between the 4th and 9th days, rarely earlier or later.

Individual Symptoms.

Fever.—The fever is very high for 1 to 2 days, reaching 39–41°C., then drops to about 38°C. and may persist from 3 to 8 days. On the average the temperature drops to 37° or even to 36°C. after 8 days and may continue to be subnormal for several days.

The relation between the temperature and the severity of the infection among those who recovered is interesting.

Recovered Cases.—There were 74 cases of yellow fever which could be analyzed. These cases came under our observation at different



TEXT-FIG. 1. 74 non-fatal cases analyzed according to defebrescence and severity of the disease. The light shaded area signifies mild, the cross-hatching moderately severe, and the solid black severe cases.

stages of the disease, ranging from the 2nd to the 8th day. The temperature in early cases (2nd, 3rd, 4th days) was usually very high, the majority being over 40°C., while those admitted later (5th to 8th days) had a temperature of 39°C. or below. The abate-

ment of fever to normal, or 37°C., occurred as convalescence was established. In the majority of instances in which the attack was mild or of moderate severity the temperature returned to normal on the 5th and 6th days. Those whose temperature returned to 37°C. on the 7th day were cases of moderate severity, though a few mild and severe cases had similar febrile reactions. The cases in which the temperature returned to 37°C. on the 8th, 9th, or 10th day were nearly all severe, and all those in which the normal was not reached until the 11th day or later were severe. The deduction from

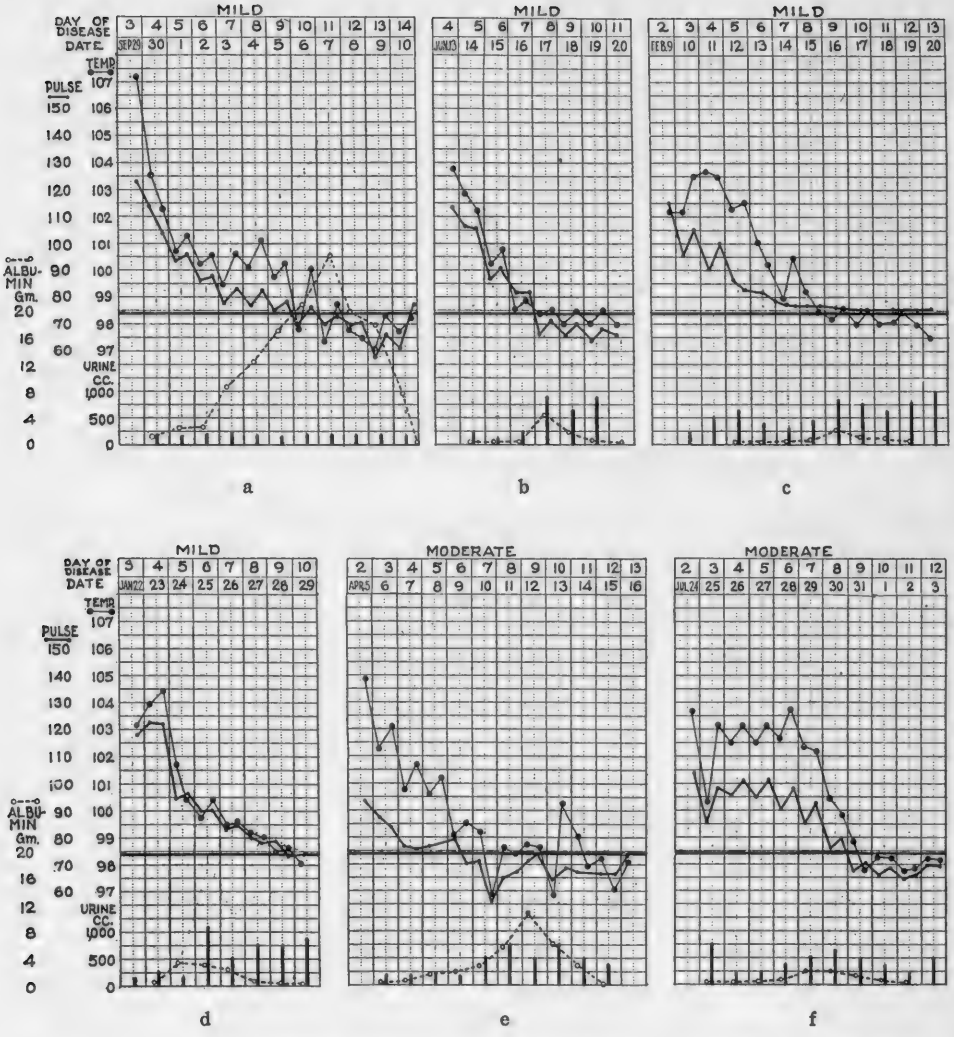
TABLE I.

Relation between Time of Defebrescence and Severity of the Disease.

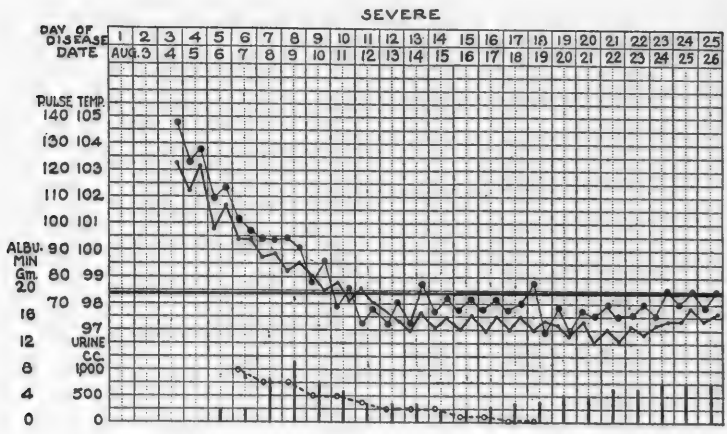
Day of disease when temperature reached 37°C.	Mild.	Moderate.	Severe.	Total.
5	3			3
6	1	5		6
7	4	3	2	9
8	5	3	8	16
9		2	10	12
10		1	12	13
11			7	7
12			5	5
13			1	1
14			1	1
15			1	1
Total.....	13	14	47	74

these observations is that the milder the attack the sooner the temperature returns to normal, and *vice versa*. In mild but undoubted cases of yellow fever the earliest date on which the normal has been attained is the 5th day of the disease (4 per cent). The bulk of the cases reached the normal on the 7th (12 per cent), 8th (20 per cent), 9th (16 per cent), or 10th (17 per cent) day. Text-fig. 1 and Table I show the distribution of cases from the standpoint of the time of defebrescence and severity of the disease.

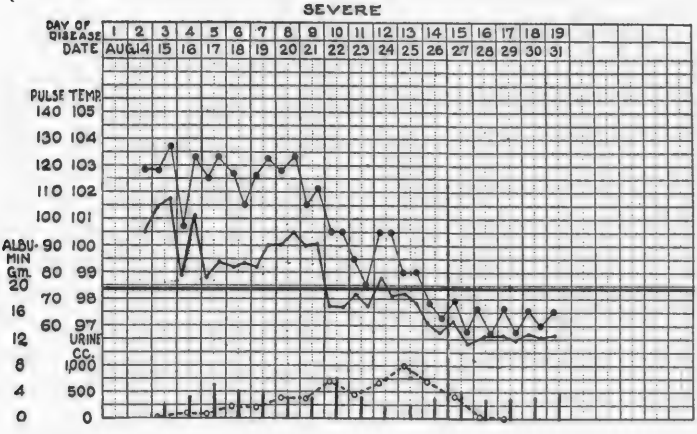
It was found that in the cases which were admitted to the hospital from the 2nd, 3rd, or 4th day of the disease there are several instances in which the temperature registered higher the following



TEXT-FIG. 2, *a* to *f*. Mild and moderate cases of yellow fever. (*a*) Case 35. Age 25 years. (*b*) Case 36. Age 21 years. (*c*) Case 37. Age 22 years. (*d*) Case 38. Age 20 years. (*e*) Case 39. Age 18 years. (*f*) Case 9. Age 19 years.



a



b

TEXT-FIG. 3, *a* and *b*. Severe cases of yellow fever. (*a*) Case 19. Age 25 years. (*b*) Case 8. Age 21 years.

day than at the time of admission or that in some instances there was a distinct remission on the 2nd or 3rd day of the disease. Again in rare instances a relatively high fever (39–39.6°C.) lasted several days. The rapidity of lysis is somewhat variable in the different cases, but the drop in the curve is rather steep. When the temperature reaches 37°C. it usually goes further down, even as low as 36°C. within a few days and may remain subnormal for several days before it attains the normal permanently. Text-figs. 2, *a* to *f*, and 3, *a* and *b* illustrate these points.

Fatal Cases.—A study of the temperature in relation to fatal cases seemed important to an understanding of the clinical features of this disease. Among the records for the year (1918) I was able to utilize 66 cases of persons dying of yellow fever (Table II and Text-fig. 4). The bulk of deaths occurred on the 5th, 6th, and 7th days, but especially on the 5th and 6th. On the 4th and 8th, 9th, and 10th days the death rates were the same, being about one-third of those for the 5th and 6th and one-half of those for the 7th day. Death seldom occurred on the 2nd or 3rd day or after the 11th day of disease.

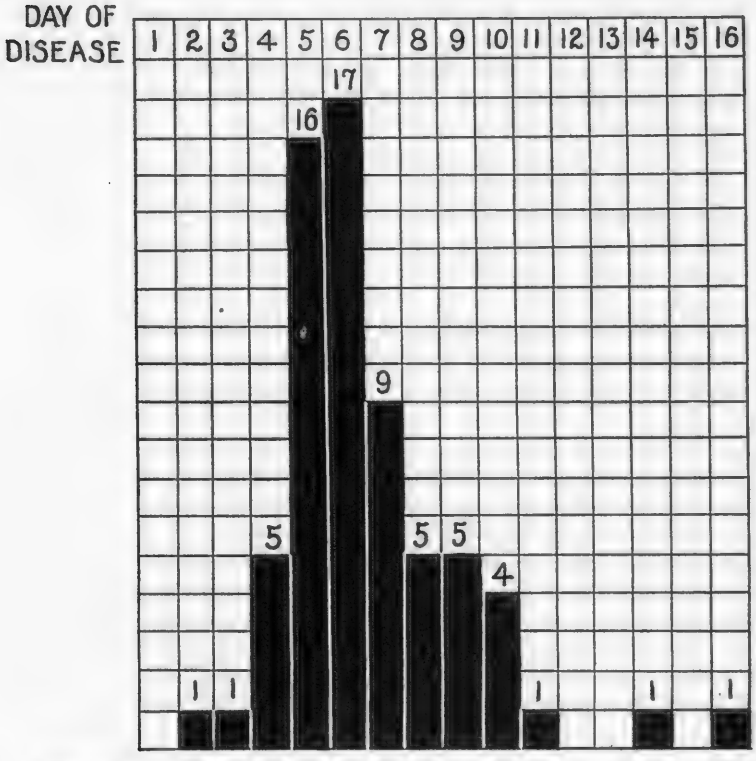
TABLE II.

Fatal Cases.

Day of disease on which death occurred.	No. of cases.
2nd	1
3rd	1
4th	5
5th	16
6th	17
7th	9
8th	5
9th	5
10th	4
11th	1
14th	1
16th	1
Total	66

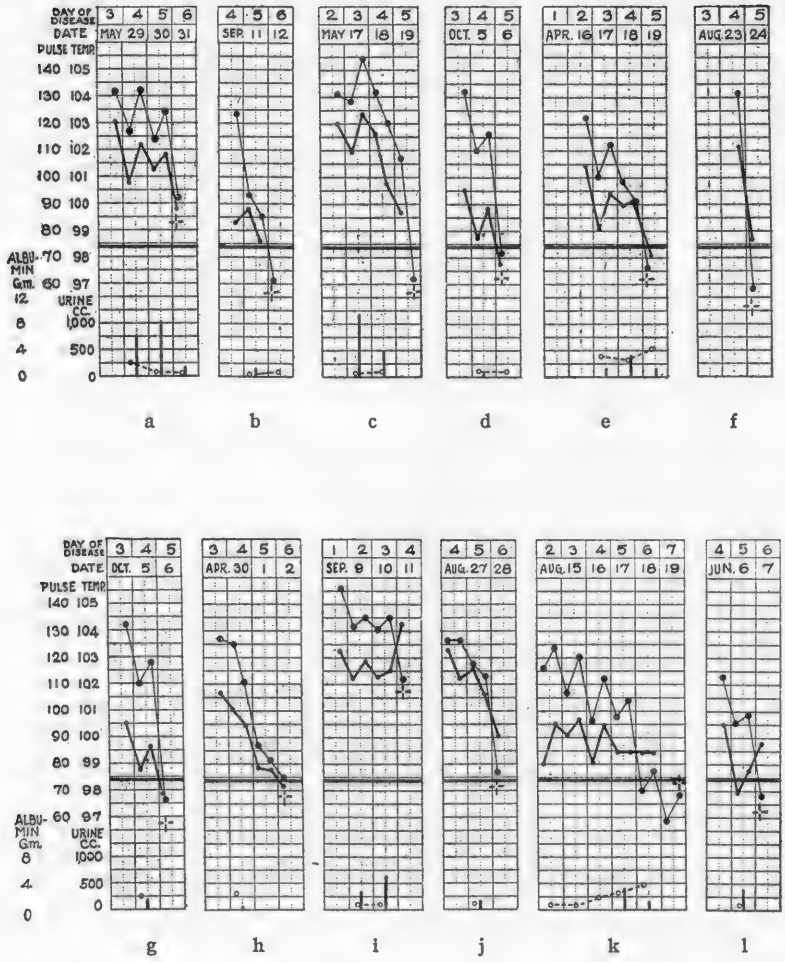
Total No. of cases.....140
No. recovered..... 74
" died..... 66 (47.1 per cent).

The temperature curves of these fatal cases (Text-fig. 5, *a* to *l*) are noteworthy as showing the rather rapid fall of temperature towards death. The initial fever in these cases was very high when observed during the early stage of the disease (above 40°C.), and some showed



TEXT-FIG. 4. The chart shows the day of disease on which death occurred in 66 fatal cases.

nearly 40°C. even on the 4th or 5th day. Death took place with a temperature as high as 39°C. in some and as low as 36°C. in others. In a few instances the temperature fell to about 37°C. or even 36° or 35°C. on the day of death but registered a sudden rise to 38°C., and in one case to 40.6°C., just before death.



TEXT-FIG. 5, *a* to *l*. Fatal cases of yellow fever. (*a*) Case 40. Age 28 years. (*b*) Case 41. Age 24 years. (*c*) Case 42. Age 20 years. (*d*) Case 43. Age 19 years. (*e*) Case 44. Age 28 years. (*f*) Case 45. Age 25 years. (*g*) Case 46. Age 18 years. (*h*) Case 47. Age 18 years. (*i*) Case 48. Age 13 years. (*j*) Case 49. Age 11 years. (*k*) Case 10. Age 26 years. (*l*) Case 50. Age 24 years.

The analysis of the febrile reaction of man to yellow fever infection becomes important in a discussion of the febrile reaction of certain experimental animals when inoculated with the blood or organ emulsions from yellow fever patients.

Disturbances in the Renal Functions.—In almost all yellow fever cases the urine contains from the 1st day of illness more or less albumin which continues and increases in quantity. On the 2nd or 3rd day granular and epithelial casts appear, being abundant in severe cases. In fatal cases the quantity of urine rapidly decreases the 1st and following days until there may be complete suppression, perhaps anuria, for 24 hours or longer previous to death. The amount seldom exceeds 500 cc. In the non-fatal cases there is a similar diminution of urine during the first 4 or 5 days, but from that time there is a gradual return to the normal output. In many cases the quantity reaches 1,000 cc. on the 8th, 9th, 10th, or 11th day, while in some the secretion may be only 500 cc. or less for many more days. The casts and albumin are less in mild cases and disappear earlier than in the severe cases, in which they may persist for from 2 to 3 weeks. They are present in largest quantity some time during the 2nd week, and as early in rare instances as the 5th or 6th day. The average maximum of albumin is about 5 gm. per liter. In a few instances it went up to 18 gm. In fatal cases the amount of albumin was not very large, being about 1 to 2 gm. per liter, although there were a few instances in which it was somewhat over 5 gm. per liter during 24 hours. In a few apparently severe cases the amount of albumin was comparatively small, 1 to 2 gm.

During the 1st and 2nd weeks of the disease erythrocytes are always found in varying quantities in the urine.

Jaundice.—This is one of the most constant manifestations of yellow fever. The intensity of jaundice is, in the majority of cases, in proportion to the severity of the disease. In a very mild case it appears later and disappears sooner than in more severe cases and may be so slight that careful attention alone will reveal its presence. In average non-fatal cases jaundice may be detected on the 3rd day of the disease, first in the scleras and then in the lighter parts of the skin. On the 4th or 5th day the jaundice deepens, and the entire body assumes a light saffron to ocher-yellow hue. In milder cases

the height of jaundice is reached within the 1st week, while in severer cases the color goes on deepening for another week until the appearance of the patient varies from a grayish green to bright yellow. In fatal cases the jaundice may be pronounced or rather slight according to the period of the disease at which death occurred. The longer the patient lives the deeper is the jaundice as a rule. Jaundice becomes more evident after death, owing to the cessation of the blood circulation.

Bile pigments are present in the blood, pericardial, pleural, peritoneal, and cerebrospinal fluid, the adipose tissues, urine, various organs, muscles, glands, the skin, and mucous and serous membranes. The cartilages are also stained. The brains are not noticeably yellow. The urine becomes brownish yellow on the 2nd or 3rd day, and the color becomes deeper as the disease advances until it reaches the color of dark greenish brown or deep brownish yellow. In the bladder of patients dying during the 1st week the urine is of brownish yellow color.

Bile pigments are readily recognizable in the serum or plasma drawn after the 3rd day and give them a deep yellowish brown color in a later stage of the disease.

Leucocytes.—As a rule there is a slight hyperleucocytosis on the 1st day of the disease, but the number of leucocytes soon returns to normal, and in a few days a marked leucopenia sets in. There were a few exceptions, however, in which marked hyperleucocytosis was maintained for several days. The differential count showed a high percentage of polymorphonuclear leucocytes.

Hemorrhages, Vascular Injection, and Herpes.—Hemorrhagic diathesis is the third cardinal symptom of yellow fever and is never absent from fully developed cases.

During life hemorrhages are manifest in various forms, such as epistaxis, hematemesis, melena, hematuria, gingival hemorrhages, subconjunctival ecchymoses, and in occasional cases subcutaneous ecchymoses and petechiæ. Epistaxis is frequent and often profuse and may be one of the early symptoms. Hematemesis begins usually in the 1st week and becomes severer in the 2nd week. The vomitus is at first yellowish brown, but soon a coffee-ground color to which the term "black vomit" in yellow fever owes its origin. It is

a mixture of mucus, blood clot, and food in a semidigested state. This symptom may be absent in fatal cases, in which the coffee-ground contents may be first found in the stomach at autopsy. In severe but non-fatal cases the black vomit may occur in the 2nd week. Melena is a concomitant of hematemesis and is usually present in all severe cases. Gingival hemorrhages from swollen gums occur in many cases and are sometimes alarmingly persistent and profuse in severe cases. Hematuria is frequently observed, and in fact the urine of yellow fever patients always contains varying quantities of the blood corpuscles when examined under the microscope. Subcutaneous petechial hemorrhages of various sizes, from that of a pin-head to that of a split pea, were observed in several cases (Figs. 1 and 2).

During the 1st week the superficial vascular system seems to be dilated, and the blood-shot appearance of the conjunctival capillaries of the eyes is one of the most constant symptoms of the disease. The patients usually are flushed in the face in the early stage.

Herpes labialis is frequently present during the 1st week of the illness (Fig. 3).

Pulse.—Relative brachycardia is another well known characteristic symptom of yellow fever and has been observed in the majority of cases in Guayaquil. In many fatal cases, however, the pulse curve went up above that of the temperature a day or two before death. This disproportion of temperature and pulse is shown in the charts elsewhere recorded in this paper. A patient with a temperature of 39.5°C. may have a pulse of 80, and during the convalescent stage as slow as 45 or 50 beats per minute.

Nausea and Vomiting.—Nausea, accompanied by anorexia, is noted from the beginning and is soon followed by vomiting. Vomiting may occur in the beginning, however, or may begin several days later. The character of the black vomitus has already been described. It often appears bilious at first.

Pains.—Intense headaches, frontal, orbital, or general, are complained of by all patients during the first 3 or 4 days. Pains in the muscles of the trunk, loins, and calves, and sometimes in the arms and thighs are observed in all cases, being extremely intense in some. They are most marked during the first 3 days; later the patient may

make no reference to them unless questioned, being preoccupied probably by the cephalalgia. Epigastralgia is usually present and is almost intolerable in some severe cases. In mild cases the epigastric region is tender to pressure. Pains in the back in severe cases may be due to the acute nephritis. The liver is palpable, enlarged, and tender to pressure. The spleen is normal. The tongue is coated, with free red tip and edge. The lungs often show slight bronchitis.

Onset and Course of Yellow Fever in Man.

In the foregoing paragraphs the clinical features which constitute the disease known as yellow fever have been set forth. By bringing together the symptoms which occur concomitantly during the course of the infection, the reader can picture to himself the appearance of a mild, moderate, severe, or fatal case. A brief resumé is given here, however.

Mild Infection.—Onset with severe headache, coated tongue with red tip and edge, suffusion of conjunctivæ, myalgia, anorexia, and nausea, accompanied by a temperature of about 39° or 39.5°C., usually without chill. The patient is rather ill, but still able to go about. There is a moderate hyperleucocytosis which presently may drop to normal or to leucopenia. The pulse is relatively slow. A trace of albuminuria and icterus are present during the next few days. All symptoms rapidly disappear within a week or a little more.

Moderate Infection.—All the symptoms just described are present, together with black vomit during the 1st week. The patient is ill enough on the 1st day to go to bed. The presence of albumin and casts in the urine, oliguria, and icterus are rather pronounced and may persist for 10 days or more, when convalescence begins. Gastralgia is present. The liver is palpable and tender.

Severe Infection.—All symptoms are much aggravated, and there may be rigor and a fever around 40°C. Epistaxis, hematemesis, hematuria, melena, and gingival hemorrhages follow. Icterus is intense, and the urine diminishes rapidly as the disease advances, and great quantities of albumin, casts, and bile pigments are contained in it. The patient may become delirious at the end of the

1st week. The epigastric pain is severe. Profuse hemorrhages, hypothermia, and exhaustion ensue. By the 12th day the urine begins to increase in volume and continues to do so for subsequent days. With the increased urine the patient begins to improve, and within another week or two all the symptoms except jaundice and albuminuria gradually disappear.

Parotitis and a secondary fever on the 14th to 16th day were observed in certain cases as complications.

Fatal Infection.—The onset of the yellow fever that ends in death is the same as that observed in the severe non-fatal cases. Both are very grave from the beginning. Some cases appear to be in a state of exaltation, with brightly flushed face and blood-shot eyes, when brought in on the 2nd day, but the bloody vomit soon begins, and the patient is rapidly seized by an agony of pains. Albumin, casts, and blood cells are present in the urine. On the 3rd or 4th day there may be total anuria. The patient soon becomes delirious, then comatose, and dies in convulsions. There may be a sudden drop in temperature before death or in some instances a sudden rise to near 40°C. When death occurs after the 6th day the temperature is already low, sometimes below 37°C. Jaundice is always present in fatal cases. In some cases the black vomit, or rather hematemesis (still quite bright red) occurs near death or may be found at autopsy.

The foregoing summary is intended only to give a very general impression, the details being recorded in other papers.

Autopsy Findings in Yellow Fever in Man.

This part of the subject is important in completing our knowledge of the disease as it affects man. All the clinical manifestations are only the apparent characteristics of the disease and are explained and extended by the pathological findings.

Postmortem rigor and lividity are pronounced. There is intense jaundice throughout the entire body. The nostrils and mouth may be partly filled with blood clots and the face smeared with blood. The region about the anus is often stained with melena. Uterine hemorrhages were observed in young women. The skin, subcutaneous tissues, and muscles are yellow.

Lungs.—Often edematous; show marked hypostasis; crepitant. In every case there were ecchymotic hemorrhages, variable in size and distribution. The size varies from a few millimeters square to the size of a split pea, and is sometimes as large as a pigeon's egg. The hemorrhagic foci are sharply defined in some and diffuse in others. If recent they are vivid red, if several days old bluish red. On section they are seen to extend deep into the substance of the lungs, some into the interior. They are discrete and multiple, and the number varies in different cases and in the two lungs.

Heart.—Often shows hemorrhagic foci in the pericardium, which contains icteric fluid and is often dilated on the right side and in diastolic condition. The myocardium is grayish yellow, brittle, and cloudy. Numerous punctiform ecchymoses are frequently found on the surface. The endocardium is clear, with occasional punctiform hemorrhages along the papillary muscles. The valves are intact.

Liver.—Hyperemic and often somewhat enlarged. The color varies, yellowish brownish red, ocher-yellow, saffron-yellow, light greenish yellow, brownish yellow. The whole may be of uniform color or shaded, blended, or minutely mottled. The yellower or greener color indicates a more advanced stage of degeneration, in which the parenchyma is brittle and tears easily when handled with forceps. In a brownish red liver the tissue still retains much of its normal consistency. Minute ecchymotic spots are sometimes visible on the surface.

Gall Bladder.—Usually full of deep greenish yellow bile. There may be multiple ecchymoses in the wall.

Kidneys.—Enlarged, highly hyperemic, and reddish yellow in color. Varying number of ecchymoses, some punctiform and some as large as a bean, may be present in the capsule, which strips easily. In a few instances punctiform hemorrhages were found in the cortex. On section hyperemia is noticed along the junction of the cortex and medullary portion. The cortex is broader and shows general swelling and cloudiness. The medulla is succulent and icteric. The renal pelvis may be free, but not infrequently it contains a blood clot, or numerous punctiform hemorrhages are seen irregularly situated. The kidneys, therefore, show acute parenchymatous inflammation.

Suprarenal Glands.—Appear to be congested and more friable than normally. In one instance an extensive hemorrhage was found in the perinephritic adipose tissue.

Gastrointestinal System.—The stomach usually contains a viscid semifluid of coffee-ground appearance, known as the black vomit or *vomito negro.* In some instances freshly extravasated blood gives the gastric contents a dark reddish hue, or they may be dark green in color with black particles. The serosa is free from any ecchymoses, but the mucosa is intensely injected with more or less numerous ecchymoses, particularly near the cardia.[3] The small intestines as well as the colon are similarly affected and contain a tarry fluid (melena).

Bladder.—Except for occasional ecchymoses on the serosa nothing special has been observed. The bladder is often full.

Uterus and Ovaries.—Intense injection of the endometrium and sometimes hemorrhage in the uterus. The ovaries are somewhat injected. In one instance there was hemorrhage of both ovaries.

Testicles.—No change found.

Spleen.—Apparently normal.

Lymphatic Glands.—Swelling and hemorrhages were observed in the bronchial, mesenteric, and other lymphatic glands. This condition, as well as the constant ecchymoses in the lungs, seems to have received little attention from previous investigators.

Serous Membranes.—Pleuræ, peritoneum, and omentum are usually free from hemorrhages.

Skin and Mucous Membranes.—Occasional petechial hemorrhages were observed.

Nervous System.—The central nervous system is macroscopically unchanged. The cerebrospinal fluid is icteric and the brain edematous. The membranes are injected.

Histological Findings.

Lungs (*Figs. 4 to 7*).—In the majority of instances areas of hemorrhage of varying extent are found and also small foci of infiltration with polymorphonuclear leucocytes. The alveoli are filled with

[3] This has been constantly observed by Dr. Pareja in his long experience.

polymorphonuclear and large endothelial cells and red blood corpuscles. The thrombi in some vessels are similar to those seen in infarctions.

Liver (*Figs. 8 to 11*).—The liver is necrotic in the large proportion of cases, and the parenchyma is full of extravasated blood. Only around the vessels are the living cells found, better preserved around the central vessel than around the portal canal. A moderate amount of pigment is sometimes found within the bile capillaries. The blood is distributed throughout the necrotic areas and not always confined to the blood vessels. Mitotic figures are met with among the liver cells. Most liver cells are vacuolated, and some are distended with vacuoles. Hemorrhagic areas are found. Numerous red-staining cells mingle with those less degenerated. There are a few granules in the liver cells but no pigment. In the region of the portal canal small foci of lymphoid and plasma cell infiltration are encountered.

Kidneys (*Figs. 12 to 15*).—The convoluted tubules are somewhat dilated with granular excretion. There are many deeply stained hyaline and granular casts. The epithelium of the convoluted tubules shows granular and somewhat vacuolated cytoplasm. The glomeruli are moderately or considerably injected; there is rather marked injection of the medulla with several hemorrhagic areas.

Stomach.—A few minute foci of infiltration with polymorphonuclear leucocytes; in certain areas a more diffuse infiltration of lymphoid and plasma cells (some bacilli). The superficial portion of the mucosa is markedly congested. The mucosa is somewhat injected in places.

Large and Small Intestine.—Injection and occasional hemorrhages.

Heart.—The muscle fibers show one or more vacuoles situated in the central portion, suggestive of fat. Certain fibers appear somewhat swollen. The nuclei are large and vesicular.

Spleen.—Numerous large phagocytes containing red cells. Follicles atrophied in one case; in another much blood in the pulp. A large number of phagocytic cells appear in certain areas. These contain red cells and red-staining granules.

Lymph Nodes.—(1) Some degeneration and phagocytosis centrally situated in the follicles. (2) Peripheral sinuses filled to a large extent

with polymorphonuclear leucocytes and a small number of phagocytic cells. The lymph follicles show small areas of degeneration centrally situated.

Pancreas.—Certain of the gland alveoli show small groups of degenerated cells. Sometimes there are no lesions.

Adrenals.—There is a marked degree of parenchymatous degeneration, affecting chiefly the medulla, and considerable congestion. In some cases the adrenals are intact.

Nervous System.—Nothing abnormal.

SUMMARY.

The clinical and pathological features of the yellow fever prevalent in Guayaquil conform with those described by other investigators of this disease as it has occurred elsewhere, both epidemically and endemically.

EXPLANATION OF PLATES.

PLATE 31.

FIG. 1. Patient Ch. The photograph, which was taken on the 5th day of the disease, shows large and small areas of subcutaneous hemorrhage on chest and arms, also on the temple. The blotch on the left breast was caused by the application by the patient of mustard plaster. The patient died on the 7th day.

FIG. 2. A patient who was severely ill but finally recovered. There were extensive subcutaneous hemorrhages all over the body of a mottled appearance but with no actual circumscribed spots.

FIG. 3. Patient Co. The photograph, which was taken on the 3rd day of the disease, shows herpes labialis, particularly of the lower lip. The patient died on the 4th day.

PLATE 32.

FIGS. 4 to 7. Sections of lung from four different cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. Hemorrhagic areas, varying in extent, can be seen in all these lesions. They are well defined with respect to the normal tissue (Fig. 4). There is a varying degree of edema. Fig. 4 is from a patient dying on the 6th day, Fig. 5 from a patient dying on the 5th day, Fig. 6 from a patient dying on the 8th day, and Fig. 7 from a patient dying on the 7th day. × 150.

PLATE 33.

FIGS. 8 to 11. Sections of liver from cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. The general character of the lesion consists in necrosis and vacuolization of the liver cells. Some groups of cells are completely disintegrated. The necrotic areas are occupied by debris and hemorrhage, particularly marked in Figs. 10 and 11. Fig. 8 is from a patient dying on the 6th day, Fig. 9 from a patient dying on the 5th day, Fig. 10 from a patient dying on the 8th day, and Fig. 11 from a patient dying on the 7th day. × 150.

PLATE 34.

FIGS. 12 to 15. Sections of kidney from cases of yellow fever, fixed with Zenker's fluid and stained with eosin and methylene blue. The general character of the lesion is the same in all these sections: swelling and degeneration of the renal epithelia in the tubules with varying degrees of hemorrhage into the connective tissue. The glomeruli are highly congested; some of the epithelia of the tubules are vacuolated and desquamated. The lumina of the tubules are filled with granular casts. Fig. 12 is from a patient dying on the 6th day, Fig. 13 from a patient dying on the 5th day, Fig. 14 from a patient dying on the 8th day, and Fig. 15 from a patient dying on the 7th day. × 150.

FIG. 1.

FIG. 2.

FIG. 3.

(Noguchi: Etiology of yellow fever. I.)

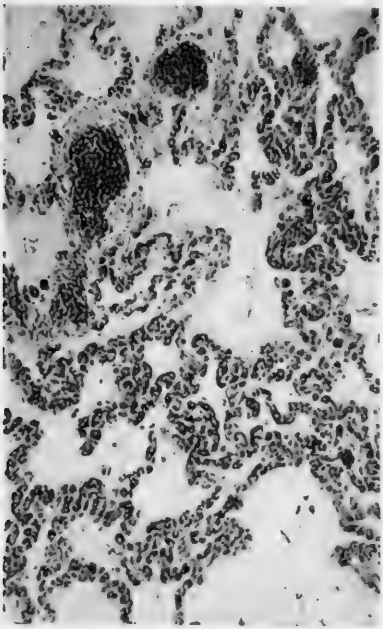


FIG. 4.

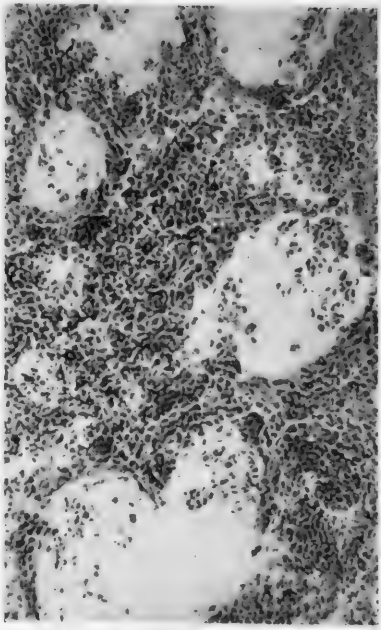


FIG. 6.

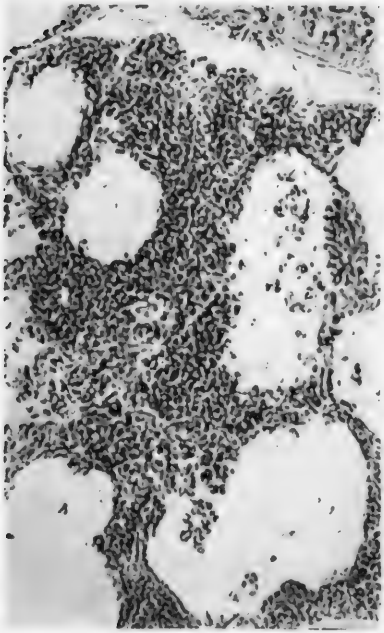


FIG. 5.

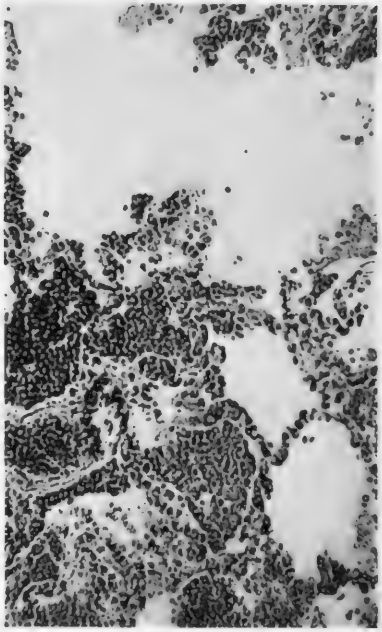


FIG. 7.

(Noguchi: Etiology of yellow fever. I.)

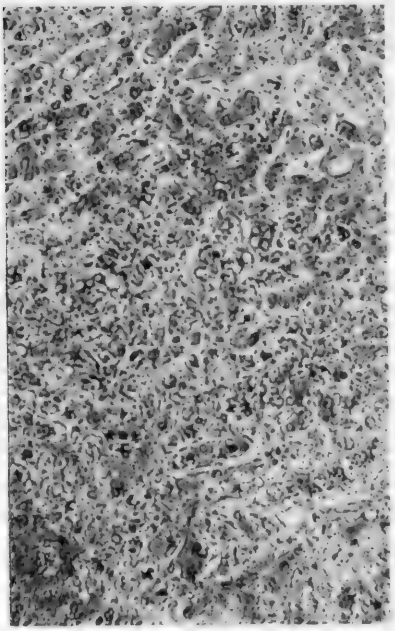


FIG. 8.

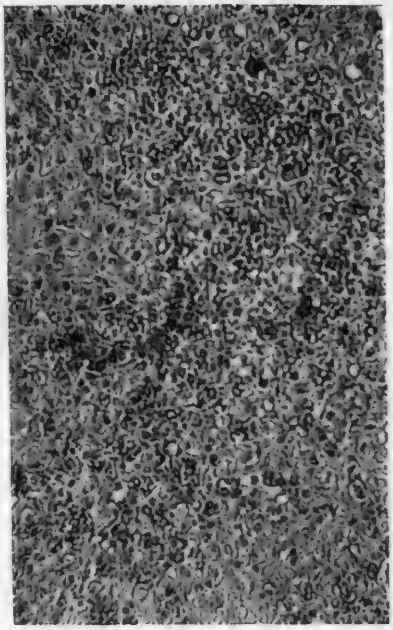


FIG. 10.

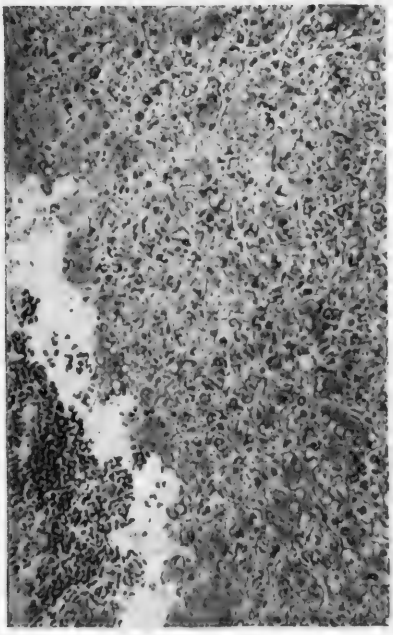


FIG. 9.

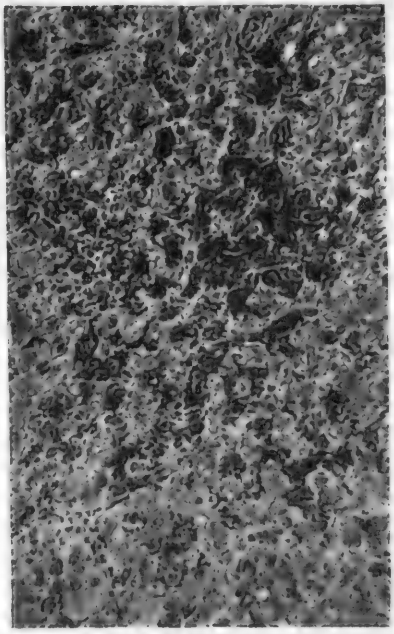


FIG. 11.

(Noguchi: Etiology of yellow fever. I.)

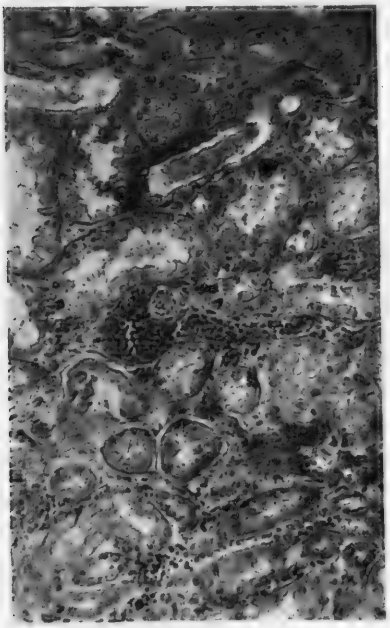


FIG. 12.

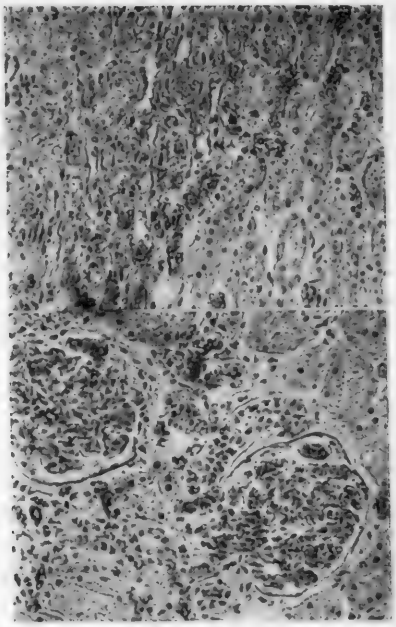


FIG. 14.

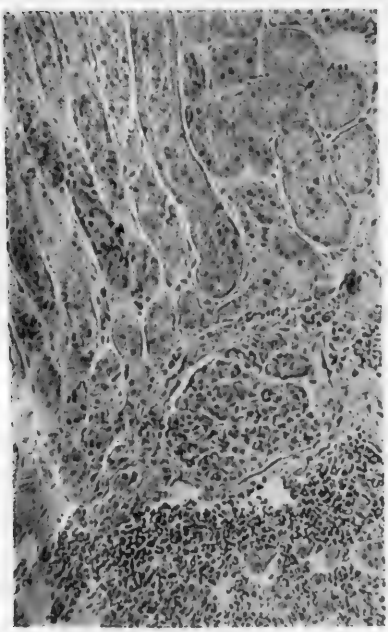


FIG. 13.

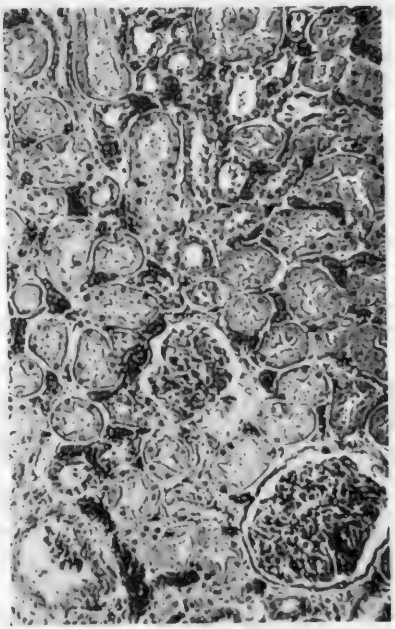


FIG. 15.

(Noguchi; Etiology of yellow fever. I.)

ETIOLOGY OF YELLOW FEVER.

II. Transmission Experiments on Yellow Fever.

By HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plate 35.

(Received for publication, March 27, 1919.)

For the purpose of transmitting yellow fever to animals experiments were made to reproduce the disease in whatever kind of animal was available by inoculation with the blood from patients admitted to the Yellow Fever Hospital in Guayaquil during my stay there. The blood was drawn from the median basilic vein of the patient at various stages of the disease, mainly during the 1st week, with a sterile Luer syringe and injected before coagulation into the peritoneal cavity (in mammals) or into the pectoral muscles (in birds). 10 cc. of blood were usually taken and distributed among from one to four animals in each instance. When more than one kind of animal was used for one patient correspondingly larger quantities of blood had to be drawn. The following animals were employed: ringtail monkey, rabbit, guinea pig, cat, dog, donkey, guatusa,[1] comadreja,[2] and ozo-melero[3] among the mammalians; pigeon, paloma de tierra,[4] bluebird, mantas,[5] blackbird, parrakeet,[6] reedbird, blancos,[7] and diostede[8] among the birds.

[1] *Dasyprocta aguti* (animal akin to rabbit).
[2] *Bassaris bassaricyon* (?) (resembling an opossum).
[3] *Cholopus didactylus* (two-toed sloth).
[4] Family of Peristerinæ (ground-dove).
[5] Siskin (probably).
[6] Family of Bolborhynchus.
[7] Similar to a magpie, but white.
[8] Genus *Pteroglossus*.

All the animals inoculated were kept under observation at least 1 month before being discarded as negative, even when no symptoms were observed. An animal which showed a rise of temperature after a period of several days was closely watched, and when more than one of the animals of the series receiving the same blood became febrile one was killed for examination and further passage made in the same species of animal. As will be seen later (Case 2) such a step was essential with some specimens of yellow fever material, as by this means alone was it possible to reproduce an experimental condition resembling yellow fever on the third animal passage.

None of the birds showed any definite symptoms when first inoculated with the blood of yellow fever patients, but upon subsequent injection of the organ emulsion of a guinea pig with the experimental yellow fever[9] parrakeets, mantas, blackbirds, reedbirds, and paloma de tierras died within 24 to 72 hours. There were no lesions except for hyperemia of the visceral organs. The pigeons, bluebirds, and toucans showed no definite reaction. This rather rapid death following the second inoculation may have been due to an anaphylactic phenomenon. Rabbits and guatusas showed a temperature of 40–41°C. on the 4th to 5th day after inoculation but became normal after 48 hours. There were no other symptoms, and the animals remained well during the period of observation (1 month). The ringtail monkeys showed a similar but more prolonged fever reaction lasting several days. The animals appeared depressed after 48 hours and refused to take food. The conjunctivæ were injected for several days, but there was no jaundice. Unfortunately the number of experiments with this animal was limited to five because of the difficulty in securing them in sufficient numbers. Donkeys showed no reaction to the injection of yellow fever blood in large quantities.

Apparently none of the animals enumerated above possesses a sufficient degree of susceptibility to enable the yellow fever virus to multiply and reproduce the symptoms and lesions known as yellow fever in man. It is possible that some of them had an extremely mild or atypical form of the infection.

[9] For the sake of brevity the condition induced by inoculation will be termed "experimental yellow fever." Further evidence bearing on the validity of this term will be presented in successive papers of the series.

Since guinea pigs could be secured more easily, and in view of the success of Inada and Ido[10] with this species in the study of infectious jaundice, they were extensively used during the present study. Guinea pigs are native to Ecuador and are found wild in the mountainous regions.[11] All except 60 of the guinea pigs which were used in these experiments (which were taken from New York) were reared in the mountains and shipped to the Yellow Fever Hospital. They stand captivity well for several months.

Experiments with Guinea Pigs.

Blood and Organ Emulsions.—Of 74 guinea pigs inoculated with the specimens of blood from 27 cases of yellow fever, 8, representing 6 cases, came down with the symptoms resembling human yellow fever. In one instance, however, the reproduction of the disease required three successive inoculations in guinea pigs. In this instance one of the animals showed a rise of temperature on the 5th day and was killed for examination and transfer on the 6th day. The liver was degenerated, and the kidneys were highly congested. A few hemorrhagic spots were found in the lungs. In a further passage of the organs to two new guinea pigs there was a similar febrile reaction with ecchymoses in the lungs but only a trace of icterus. On transfer of the second passage material typical hemorrhages, icterus, and albuminuria were induced. With the materials from only one of several autopsies was a positive transmission obtained. The details of the transmission experiments follow.

The first positive transmission was obtained in the case of Patient A.

Case 1 (*Text-Fig. 1, a*).—A. A., female, age 17 years; servant, native of Latacunga (9,055 feet above sea level). Had come to Guayaquil 3 months previously.

Onset, July 14, 1918. Headache, chills, fever, and severe pains throughout the body. July 15. Vomited chocolate-colored matter and felt pains in epi-

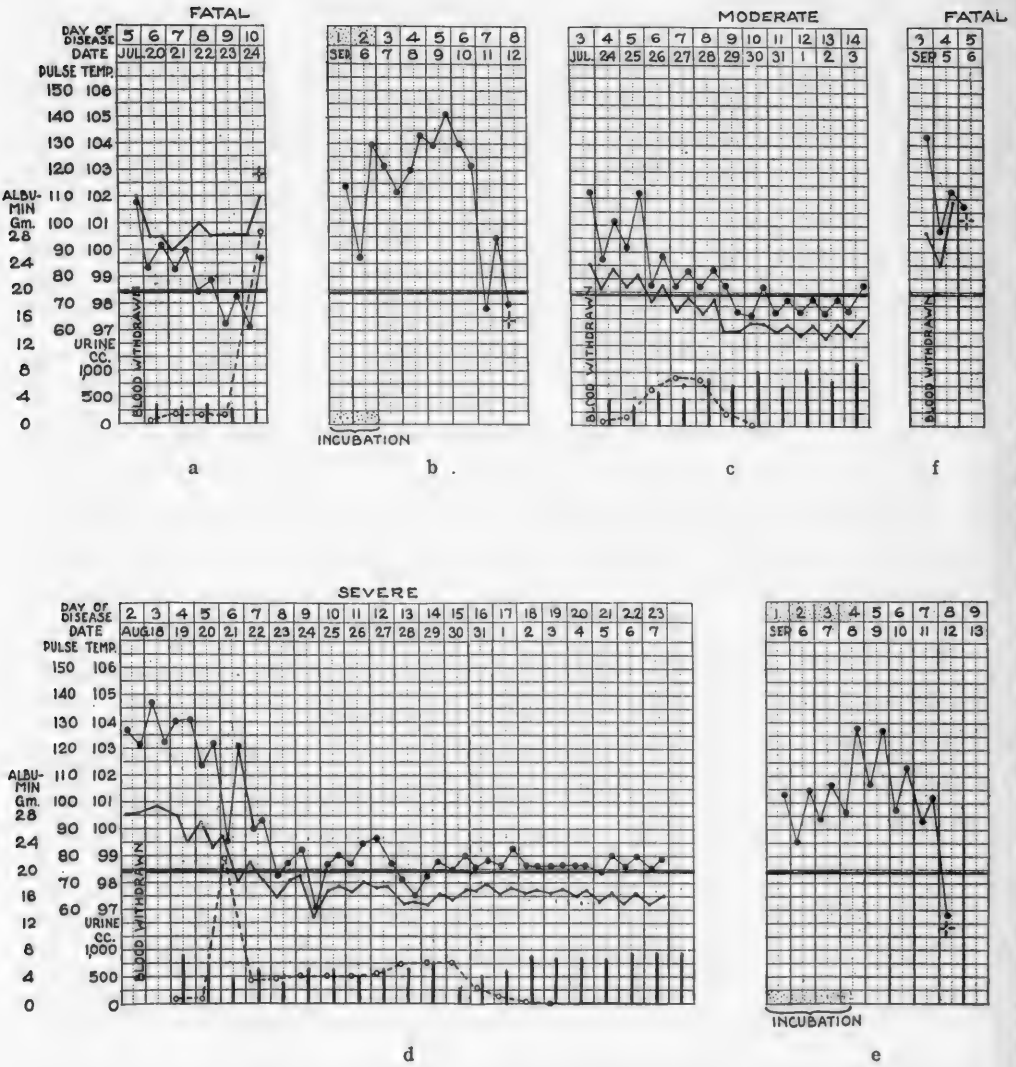
[10] Inada, R., Ido, Y., Hoki, R., Kaneko, R., and Ito, H., *J. Exp. Med.*, 1916, xxiii, 377.

[11] Guinea pigs are in the market for food in Ecuador, but the municipality of Guayaquil has forbidden their being kept in the city on account of the possibility of spreading bubonic plague.

gastric region. July 19. Admitted to hospital. Was very sick and vomited dark bloody matter. Conjunctivæ intensely injected and distinctly jaundiced. Liver tender but not palpable. General jaundice of the skin. Blood drawn from median basilic vein into citrate solution and inoculated into guinea pigs immediately and also after 3 days cultivation at 25°C. July 20. Patient felt better, but gums began to bleed. Abdominal pains not marked. July 22. Condition worse. Patient very ill, vomited constantly ("coffee-grounds vomitus"). Jaundice very marked. Liver tender and swollen. Tumefaction of thighs because of menstruation. July 23. Delirium and insomnia with intense epigastric pains. July 24, 9 p.m. Died.

Autopsy.—Performed 11 hours post mortem. Well nourished young woman, with well marked rigor mortis and lividity. Body cyanotic and mottled. Pronounced general jaundice. Mouth and nostrils contained dark, partly dried blood clots, and face was badly smeared with dried blood. Similar blood specks were found on the hands and the genital regions, the skin of the thighs being extensively excoriated owing to hemorrhages from the uterus. No petechiæ or subcutaneous ecchymoses present. Lungs edematous. Pronounced hypostatic congestion of the dependent parts. Several ecchymoses of pea size present. Pleura free, containing moderate amount of deeply jaundiced clear fluid. On section the hemorrhagic spots of the lungs were seen to extend many centimeters into the parenchyma; some occult foci of similar ecchymoses in the interior. Some old and some new foci of tuberculosis in the upper lobes. Pericardium apparently free from any changes; scanty icteric fluid in the cavity. Heart normal in size; in diastolic state, containing fluid blood in right heart. Few minute ecchymoses on the surface. Muscles pale, flabby, and brittle. Endocardium and valves normal except for a few petechial hemorrhages on the papillary muscles. Liver pale ocher-yellow in color, fatty, and streaked with congested areas. Color on section uniformly yellow; parenchyma friable. Stomach distended with gas, containing blackish bloody mucous material; mucosa hyperemic, especially a few minute hemorrhagic spots near the cardia. Intestines filled with dark tarry contents; mucosa hyperemic. Kidneys swollen and hyperemic, capsules non-adherent; on section numerous ecchymoses in the pelvis; increase of the cortical layer and cloudy swelling. Deeply jaundiced. Suprarenal glands hyperemic. Spleen and pancreas apparently normal. Ovaries congested and uterus hyperemic, containing extravasated blood in the cavity. Small amount of deeply yellowish urine in bladder.

Animal Inoculation.—The blood, drawn on the 5th day of disease, was used in two different ways. One portion was immediately inoculated into the peritoneal cavity of two guinea pigs, and the other was put into a mixture of Ringer solution 3 parts and human serum 1 part with 0.3 per cent neutral agar, then well mixed and covered with a layer of paraffin oil. This semisolid mixture was placed in a thermostat at room temperature for 3 days (20–25°C.) and then injected into two guinea pigs intraperitoneally. The amounts of the original blood injected into each guinea pig were from 2 to 3 cc. The protocols follow.



TEXT-FIG. 1, *a* to *f*. Cases of yellow fever in which positive transmissions to guinea pigs were obtained. (*a*) Case 1. Age 17 years. (*b*) Guinea Pig 129 A. Strain, Case 1. (*c*) Case 2. Age 23 years. (*d*) Case 3. Age 20 years. (*e*) Guinea Pig 353 C. Strain, Case 3. (*f*) Case 4. Age 15 years.

Guinea Pig 50.—July 19, 1918. Inoculated with 3 cc. of the fresh blood intraperitoneally. The temperature rose to 39.6°C. on the 6th day, dropped to 38.5° on the 7th, again rose to 39.8° on the 8th, was 40° on the 9th, and 39.7° on the 10th day. The animal became less active, and on the 11th day it had a temperature of 39.1°, which continued for 3 days and then returned to normal (38–38.5°). On the 11th day the scleras appeared icteric, and the capillaries of the conjunctiva much suffused. The animal recovered completely in 15 days. On a subsequent injection (Aug. 10, 1918) with material taken from other guinea pigs inoculated with the enriched blood of the same patient this animal failed entirely to react.

Guinea Pig 51.—July 19, 1918. Inoculated intraperitoneally with 2 cc. of the same lot of blood. The temperature was 39.9°C. on the 9th day, 40.5° on the 10th, and 39.2° on the 11th, returning gradually to normal (38.5–39°). There was a suspicion of jaundice on the scleras on the 12th day which disappeared in a few days. This animal remained well and was subsequently (Aug. 10, 1918) tested against material taken from this case. It failed to react.

Guinea Pig 80.—July 22, 1918. 4 cc. of the mixture of the citrate blood and the culture medium, which had been incubated for 3 days, were injected intraperitoneally into the guinea pig. The temperature was 40°C. on the 5th day, 40.1° on the 6th, and 38° on the 7th, when the animal became distinctly jaundiced. Death occurred the same day (July 29).

Autopsy.—Marked general jaundice. No definite subcutaneous ecchymoses. Dried blood specks around the nostrils. Mucous membranes very yellow. Lungs congested; showed few disseminated ecchymotic spots which on section were seen to extend deeper into the parenchyma. The size varied from a minute point to that of a split pea, and the color from bright red to dark bluish red. The contour of these areas was usually sharply demarcated from the normal tissue. Lungs edematous and the lower lobes highly hypostatic. Pleuræ not affected. Right heart appeared dilated and contained semicoagulated cyanotic blood. A few punctiform hemorrhages on the anterior surface. Muscles friable. Liver yellowish brown in color and mottled with areas of congestion. The markings of the lobules were prominent. Consistency firmer and less succulent than the normal. Stomach distended with undigested food partly tinted with the dark blood which escaped from the hemorrhagic spots on the highly congested mucosa, especially marked near the cardia. Intestines highly hyperemic and showed numerous spots of hemorrhage along the mucosa. Contents bloody. Kidneys intensely hyperemic and more or less swollen, showing a few punctiform hemorrhages in the cortex. On section the pelvis was filled with blood and there were some ecchymoses. The cortex was broad and red, and the medulla succulent and cloudy. Adrenals hyperemic and swollen. Spleen, pancreas, bladder, and testes apparently unaffected. Along the abdominal wall the muscles were congested and showed some disseminated ecchymoses.

Microscopic Examination.—The blood was apparently free from any microorganism when examined under the dark-field microscope. Emulsions were

prepared from the liver, kidney, and adrenals for examination by dark-field and also for further passage. A very few actively motile organisms belonging to the genus *Leptospira* were demonstrated in the liver and kidney emulsions but not in the adrenal.

Passage.—Two guinea pigs were immediately inoculated with a mixture of the emulsions of the liver and kidney from Guinea Pig 80 and a further generation of this strain was obtained in the two following guinea pigs.

Guinea Pig 80 A.—July 29, 1918. The organ emulsion (2 cc.) was inoculated intraperitoneally. The animal showed a temperature of 41.3°C. on the 4th day, 40.6° on the 5th, and 38.7° on the 6th, when the color of the skin and mucosa became intensely icteric. Death occurred at 3 p.m. on Aug. 3 while the animal was being placed in a mosquito cage with the stegomyias.

Autopsy.—The general appearance was the same as that of Guinea Pig 80. The urine contained bile pigment, albumin, and abundant casts. The leptospira could not be demonstrated either in the blood, liver, or kidney.

Guinea Pig 80 B.—July 29, 1918. The emulsion was applied to the scarified surface of the skin. The highest temperature (morning) was 39.9°C. on the 7th day. It went down to 38.8° on the 9th day, when the animal became very yellow. The animal died on the 10th day (Aug. 7).

Autopsy.—About the same as in the foregoing experiment except for a higher degree of degeneration of the liver, which was ocher-yellow, exactly as in some human cases. No leptospiras were demonstrated by the dark-field microscope, but a further successful transfer was made.

Guinea Pig 81.—July 22, 1918. 5 cc. of the enriched blood of Patient A. were injected intraperitoneally. The temperature was 40.3°C. on the 6th day, 40° on the 7th day, 39.9° on the 8th, and 38.7° on the 9th day, when jaundice became noticeable. The animal was found dead on July 31.

Autopsy.—Lesions similar to those already described in Guinea Pig 80. The stomach was filled with a semifluid blackish gray matter (digested blood). Dark-field examination of the blood, liver, and kidney failed to demonstrate any organism. Transfers were made to six guinea pigs. The protocols are given below.

Passage.—July 31, 1918. Six guinea pigs were inoculated with the emulsions of the kidney and liver, intraperitoneally and also percutaneously after depilation.

Guinea Pig 124.—July 31, 1918. Kidney emulsion (1 cc.) intraperitoneally. The animal had a temperature of 40.1°C. on the 4th, 40.5° on the 5th, 39.6° on the 6th, and 39.9° on the 7th day. It continued to show high temperature for 14 days, but jaundice was not present at any time. It was discarded after 30 days (Aug. 30).

Guinea Pig 125.—The same material was used as in the foregoing experiment. The animal had a temperature of 40°C. on the 4th and 40.1° on the 5th day. After that it was 39.5° or less until the 11th day, when the animal became quite icteric. It collapsed while being used for infecting stegomyias in a cage (Aug. 10,

4 p.m.), and was killed for examination of blood, liver, and kidney. Considerable numbers of leptospiras were found in the blood and organs. Further transfers were made with success. A culture was obtained from the heart's blood, but within a week a fungus contamination destroyed it.

Passage. Guinea Pig 126.—July 31, 1918. The liver emulsion (1 cc.) of Guinea Pig 81 was inoculated into the peritoneal cavity. The temperature went up to 40°C. on the 5th, 6th, and 9th days; the animal showed definite jaundice on the 10th day and died on the 11th. The chief symptoms were all very marked.

Guinea Pig 127.—This was a duplicate of the foregoing experiment. The animal died of a secondary infection within 3 days.

Guinea Pig 128.—Percutaneous inoculation with a mixture of the liver and kidney emulsions of Guinea Pig 81. The animal had a temperature of 40°C. during the 10th, 11th, and 12th days, but it had normally registered as high as 39.5°. There was a trace of jaundice on the 13th day, which soon disappeared. The animal remained well for 31 days and was discarded as negative.

Guinea Pig 129.—This was a duplicate of the foregoing experiment. Temperature 40.3°C. on the 7th and 40.1° on the 8th day, when a suspicion of jaundice appeared. It was killed on the 8th day in order to ascertain the lesions and obtain material for culture and transfers.

Autopsy.—Jaundice too slight to be definitely recognized. The lungs, however, showed the beginning of ecchymotic spots, and the liver and kidneys were highly congested. Dark-field examination failed to reveal any organism. Transfers were made to two guinea pigs on the same day (Aug. 7). One of them died with mild but typical lesions, while the other completely recovered after having shown a temperature of 40° and 40.4°C. on the 7th and 8th days.

Text-fig. 1, *b* is the chart of Guinea Pig 129 A, which succumbed to the intraperitoneal inoculation of 0.5 cc. of a culture of this strain (Aug. 27–Sept. 5, 1918), with typical symptoms and lesions. The leptospira was demonstrated in the liver.

The foregoing experiments are of great interest as they indicate that the microorganism which was responsible for the disease and subsequent death of this patient was apparently successfully transmitted to the guinea pig. The failure of the unmodified blood to reproduce the disease in guinea pigs as contrasted with successful transmission by means of the same blood after a temporary enriching of the virus *in vitro* may be explained by the fact (as subsequent experiments concerning the properties of the serum of yellow fever patients and the properties of the organism indicate) that the inoculation of the fresh blood carried with it a certain amount of antibodies antagonistic to the development in the body of the guinea pig, while this

property is certain to be considerably modified when incubated in the culture medium employed. Moreover, as the organism is capable of multiplying outside the body at room temperature there is temporary enrichment of the organisms before inoculation into the guinea pig.

It is also shown in this experiment that the microorganism is variable in virulence, being able to produce a fatal infection in some, an abortive infection in others, and in still others no infection at all. The organism is capable of entering the body of the guinea pig in some instances by the smearing of the infective material. That there is an abortive form of the infection is shown by the immunity that it confers upon the guinea pigs which survived such an infection. The infective agent may be passed from one guinea pig to another by timely transmission.

A leptospira has been found to be associated with this case, but its demonstration by means of the dark-field microscope is not always successful; it may or may not appear in a subsequent passage. That this organism, notwithstanding the difficulty of demonstrating it, is etiologically related to the disease known as yellow fever in Guayaquil is made highly probable by subsequent experiments.

Fig. 1 shows the organism in the blood of a guinea pig inoculated with the culture of this strain.

The second positive transmission was obtained in the case of Patient P.

Case 2 (*Text-Fig. 1, c*).—C. P., male, age 23 years; tailor, robust, a native of Cuenca (highland). Had been in Guayaquil a month.

Onset July 20, 1918. Felt fever without chills; severe headache, photophobia, muscular pains throughout body, cramps in legs. No nausea. July 23. Admitted to hospital. Marked injection of the conjunctiva; slight jaundice; much albumin in the urine. Liver small but tender (Dr. Elliott). Temperature 38.9°C. Blood was drawn from the median basilic vein and injected into two guinea pigs (see protocols). July 24. Epistaxis in the morning. July 25. Nauseated easily; diarrhea. Little urine since yesterday. July 26. Jaundice advanced. Albuminuria +++. July 31. Improving. Temperature normal. Aug. 2. Trace of jaundice. No albumin in the urine. Aug. 3. Discharged.

Animal Inoculation. Guinea Pig 88.—July 23, 1918. Intraperitoneal injection of 4 cc. of the blood of Patient P. (4th day of disease). Temperature rose to 39°C. on the 4th and 40° on the 5th day. The animal was killed on the 6th day for further passages into two guinea pigs.

Autopsy.—Lungs showed a few hemorrhagic spots. Liver congested and somewhat yellowish (fatty). Kidneys congested and cloudy, showing swelling on section. Gastrointestinal system hyperemic but showing no hemorrhages. Spleen normal.

First Passage. Guinea Pig 88 A.—July 28, 1918. Received 1 cc. of blood of Guinea Pig 88 intraperitoneally. The temperature rose to 39.9°C. on the 7th day; killed on the 10th day for examination and transfer.

Autopsy.—Slight icterus. The lungs were spotted with a few ecchymoses. Liver congested and perhaps fatty. Kidneys congested and showed cloudy swelling on section. Intestine hyperemic and showed scattered hemorrhagic foci.

The liver and kidneys were emulsified and used for inoculating two normal guinea pigs (see second passage). Leptospiras not found.

Guinea Pig 88 B.—Duplicate of No. 88 A. This animal showed a slight rise of temperature in 4 days, but during observation for further evolution of the reaction it became normal and was discarded on Aug. 22.

Second Passage. Guinea Pig 89 A.—Aug. 6, 1918. 1 cc. of the mixed emulsion of the liver and kidney of Guinea Pig 88 A was given intraperitoneally. The temperature went up to 40.9°C. on the 3rd, 39.6° on the 4th, and 40° on the 5th day, but fell to 38.6° on the 6th day. Epistaxis and icterus. The animal was found dead on the 7th day.

Autopsy.—Extremely jaundiced. Lungs hemorrhagic; liver yellowish; kidneys congested, with some ecchymoses. Stomach and intestines contained blackish matter and numerous hemorrhagic areas were present on the mucosa. Leptospiras were demonstrated in the liver and kidney but not in the blood. Transfers to two normal guinea pigs were made, but this strain was lost because of intercurrent infection.

Guinea Pig 89 B.—Duplicate of No. 89 A. This animal showed less fever than usual in the 1st week but 40°C. on the 10th day. It was kept under observation until Aug. 22, but nothing further developed.

Guinea Pig 89.—This was one of the two guinea pigs inoculated with the blood of Patient P. on July 23. This animal showed a temperature of 39.3°C. on the 4th, 39.9° on the 5th, 39.2° on the 6th, 38.9° on the 7th, 39.4° on the 8th, and 39° on the 9th day. It was found dead on the 10th day.

Autopsy.—Congestion of the lungs, liver, and kidneys, but no icterus. No transfer was made.

The third positive transmission was obtained with the blood of Patient G.

Case 3 (*Text-Fig. 1, d*).—M. G., male, age 20 years; servant, robust, a native of Ambato (8,435 feet above sea level); had been in Guayaquil 2 months.

Onset Aug. 16, 1918, 10 a.m. General pain and aching; headache; fever without rigor; no nausea, vomiting, or bleeding. Aug. 17. Admitted to hospital. Eyes injected, face flushed; much prostrated. Pains in the head and limbs were

intense. Albuminuria slight; no casts. Temperature 39.9°C. Blood taken for transmission experiments. Aug. 18. Albuminuria increasing; temperature 40.3°. Condition worse. Total leucocytes 9,200 (Dr. Elliott). Aug. 19. Albuminuria increasing and numerous casts in the urine. Temperature still 40°C. Aug. 21. Delirious; otherwise condition the same. Aug. 22. No urine obtained; gums bleeding; jaundice definite; remission in fever. Aug. 23. Decided improvement; temperature going down. Aug. 26. Beginning hematuria; increased icterus. Aug. 29. Continued hematuria; great prostration. Aug. 30. Hematuria diminished; general improvement. Sept. 1. Continued improvement; cessation of hematuria. Sept. 2. Continued improvement; intense jaundice. Sept. 3. Slight pain in bladder. Sept. 7. Discharged.

Animal Inoculation. Guinea Pig 351.—Aug. 17, 1918. 3 cc. of the blood of Patient G. (2nd day of disease) were given intraperitoneally. The animal was kept under observation until Sept. 7 but remained without any noticeable reaction except that its temperature rose to 40.1°C. on the 18th day. Discarded as negative.

Guinea Pig 352.—Aug. 17, 1918. 5 cc. of the same specimen of blood were given. Temperature 40°C. on the 7th day, 39.8° on the 8th, and 39.6° on the 9th, but later returned to normal (38.6° for this animal). Tested for immunity on Sept. 7 against the A. strain and found to be resistant.

Guinea Pig 353.—Aug. 17, 1918. 6 cc. of the blood from Patient G. were inoculated intraperitoneally. The temperature rose to 40°C. on the 7th and 39.6° on the 8th day, but dropped to 38° on the 9th day. The animal was markedly icteric and was killed for examination of the blood and organs as well as for transfer and cultivation of the virus.

Autopsy.—The lungs showed a moderate number of ecchymoses. Liver light yellowish brown in color. Kidneys showed acute parenchymatous nephritis; no ecchymoses. Stomach contained a few hemorrhagic foci near the cardia with blood-stained contents. Intestines hyperemic but with few hemorrhages. Intense jaundice everywhere. Spleen normal in appearance. After a long search a few leptospiras were discovered, but the number was so extremely small that preliminary examination revealed none. Transfers were made successfully to two normal guinea pigs, and cultures were also obtained. The organism was demonstrated in the blood of guinea pigs inoculated in later experiments with this strain (Fig. 2).

First Passage. Guinea Pig 353 A.—Aug. 26, 1918. Inoculated with 1 cc. of the blood of Guinea Pig 353. Except for a slight rise in temperature nothing happened during the period of observation (up to Sept. 10).

Guinea Pig 353 B.—Duplicate of the foregoing experiment. This animal came down with typical symptoms and lesions on Sept. 5, 1918; that is, on the 10th day after the transfer.

Second Passage. Guinea Pig 353 C.—Sept. 5, 1918. Inoculated with the emulsion of liver of Guinea Pig 353 B. The course of the infection was similar to that of the positive instance, with the temperature as shown in Text-fig. 1, *e*.

The fourth positive transmission was obtained with the blood of Patient Co. Successful transmission was also made with the liver emulsion of this patient, as described below.

Case 4 (*Text-Fig. 1, f*).—J. Co., male, age 15 years; servant, native of Cuenca (highland). Had been in Guayaquil a month and a half.

Onset Sept. 2, 1918. Headache and pains in waist and legs; fever. Sept. 4. Admitted to hospital. Face flushed; conjunctiva injected and slightly jaundiced; gums reddish and swollen; tongue coated, with red tip and edge. Herpes labialis present.[12] No epigastric pain. Blood drawn for inoculation. Sept. 5. Passed no urine, but appeared calm. Increased jaundice. In the afternoon became excitable, anxious, complained occasionally of dyspnea. Melena. Sept. 6. Coma began previous night. Black vomit. Complete anuria. Death occurred at 9 a.m.

Autopsy.—A partial autopsy within 1 hour after death. Liver highly degenerated and yellow. In the emulsion a few leptospiras were found. Kidney congested, cloudy swelling. No leptospiras.

Animal Inoculation. Guinea Pig 435.—Sept. 4, 1918. Intraperitoneal injection of 3 cc. of the blood of Patient Co. (3rd day of disease). The animal showed a temperature of 39°C. on the 8th, 40° on the 9th, 39.9° on the 10th, and 39° on the 11th day, but subsequently a normal temperature. It was discarded as negative after 25 days.

Guinea Pig 436.—Duplicate of the foregoing experiment. 5 cc. of the blood injected. The temperature rose to 39.9°C. on the 9th and 40.1° on the 10th day, coming down to 38.2° on the 12th day. The animal showed distinct icterus on the 11th and 12th days and died during the night of the latter.

Autopsy.—General jaundice. Hemorrhages in the lungs and gastrointestinal tract. Liver brownish yellow. Kidney highly congested; showed cloudy swelling on section. Other organs normal in appearance. Leptospira found under the dark-field microscope and in stained slides (Fig. 3).

The fifth positive transmission was obtained with the emulsion of the liver of the same patient, who died on the 5th day. The material was taken from the body within 1 hour, and the emulsion made with Ringer's solution was inoculated intraperitoneally into four guinea pigs, the quantity for each being 1 cc. A few distorted immobile leptospiras were demonstrated in this emulsion under the dark-field microscope.

Guinea Pigs 444 and 446.—Sept. 6, 1918. Received 1 cc. of the liver emulsion from Patient Co. Died on the night of the following day.

[12] See Fig. 3, Paper I.

Guinea Pig 447.—Duplicate of the foregoing experiment. This animal never showed a reaction and died from an intercurrent infection after 22 days.

Guinea Pig 445.—Duplicate of the foregoing experiment. The animal had a temperature slightly above 39°C. for 6 days which rose to 39.9° on Sept. 13 and to 39.7° on the following day. Sept. 15. The animal was distinctly icteric and was killed for examination and transfer.

Autopsy.—Typical lesions in the lungs, stomach, liver, and kidneys. The blood, as well as the liver and kidney, showed a few leptospiras.

Passage.—From this animal the strain has been successfully passed through many generations. A culture also was obtained from a later passage.

The sixth positive transmission was obtained with the blood of Patient Ch., drawn from the median basilic vein on the 5th day of the disease and 48 hours previous to his death. In this instance 2 cc. of the fresh blood were directly inoculated into the peritoneal cavity of each of the two guinea pigs.

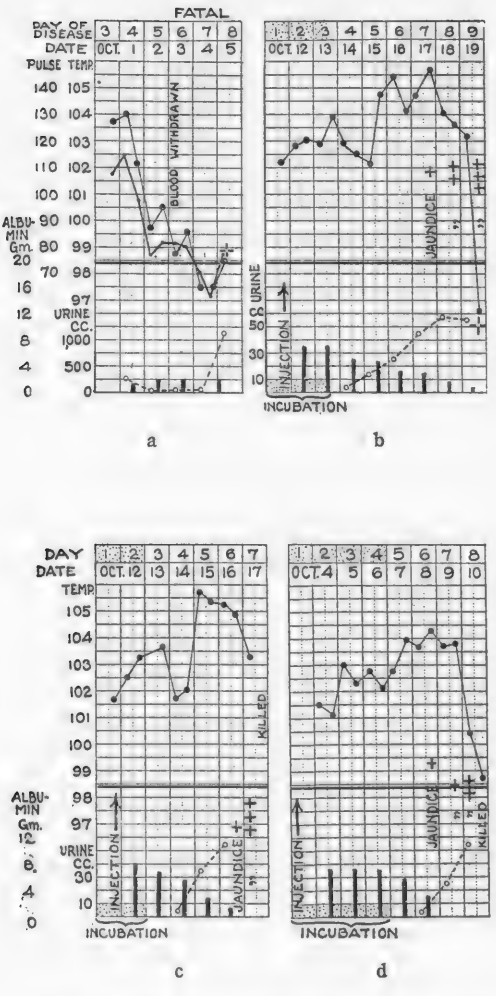
Case 5 (*Text-Fig. 2, a*).—E. Ch., male, age 30 years; well built man, a native of Guamote (highland). Had lived in Guayaquil for 8 months.

Onset Sept. 28, 1918. Sudden fever with chills, headache, pains in the limbs and back. Admitted to hospital on Sept. 30. Still complained of pains in the body and headache, with injection of the conjunctivæ; tongue coated, with free edge and tip; gums swollen. Albumin and casts in the urine, which was diminished in volume. Oct. 1. Scleras and skin somewhat yellowish. Oct. 2. All symptoms aggravated. Oct. 3. Chest showed several pea-sized hemorrhagic spots, slightly raised. Extensive ecchymotic areas were found on the skin where the patient had applied mustard.[13] Jaundice marked; gums bleeding. Frequent emission of black vomit. The blood was transmitted to guinea pigs at 2.30 p.m., Oct. 4; condition becoming worse; anuria. Oct. 5, 3 p.m. Died.

Autopsy.—Performed 2 hours after death. Intense jaundice throughout the entire body. Skin over the chest showed numerous dark hemorrhagic spots. Lungs much congested, showing many hemorrhagic spots. Liver bright yellowish in color, friable, and fatty. Kidneys highly congested, with cloudy swelling; some small ecchymotic foci in the pelvis. Capsules not adherent. Stomach and intestines hyperemic with blackish bloody contents. Heart showed a few ecchymoses in the pericardium and endocardium; muscles degenerated. Bladder contracted with small amount of dark yellow urine. Spleen, pancreas, suprarenal glands, and testes apparently unchanged.

Animal Inoculation. Guinea Pig 790 (*Text-Fig. 2, d*).—Oct. 3, 1918, 2.32 p.m. Injected intraperitoneally with 3 cc. of blood from Patient Ch., on the 6th day of disease. Temperature rose to 40.2°C. on the 6th and 39.8° on the 7th day

[13] See Fig. 1, Paper I.



TEXT-FIG. 2, *a* to *d*. Direct transmissions from patient to guinea pig by means of injection of the blood of Patient Ch., Case 5. (*a*) Case 5. Age 30 years. (*b*) Guinea Pig 790 B. Strain, Case 5. (*c*) Guinea Pig 790 A. Strain, Case 5. (*d*) Guinea Pig 790. Strain, Case 5.

and came down to 37° on the 8th day, when the scleras and skin were intensely jaundiced. The animal was rapidly weakening and was killed for examination of the lesions and also for cultivation of material.

Autopsy.—Epistaxis; hemorrhagic lesions in the lungs and gastrointestinal tract. Liver pale yellow and fatty, and kidneys and suprarenal glands congested and swollen. Other organs apparently unchanged. In the blood and emulsions of the liver and kidneys the leptospiras were demonstrated. A pure culture was obtained from the blood of this animal.

Guinea Pig 791.—Duplicate of the foregoing experiment. The animal did not become infected.

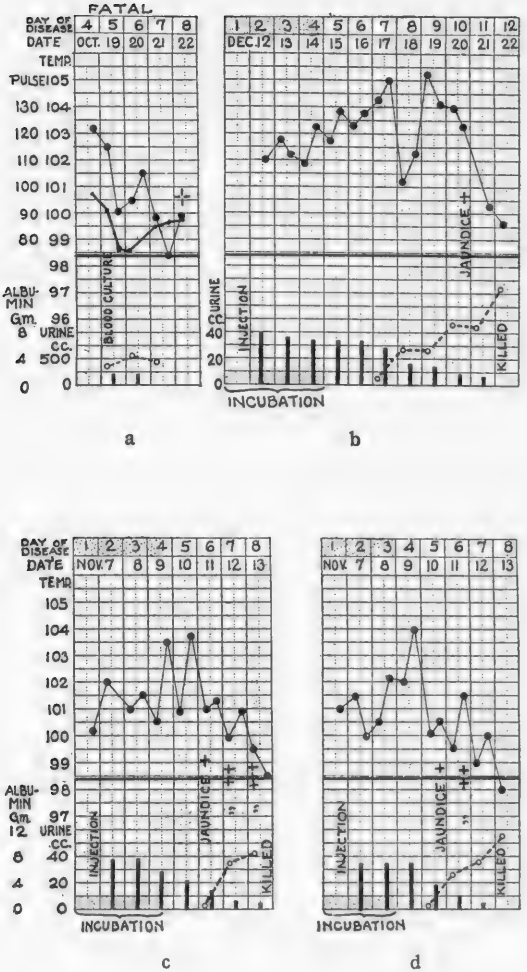
Passage.—Two guinea pigs were inoculated intraperitoneally on Oct. 11 with 1 cc. of blood from Guinea Pig 790, and both came down with typical symptoms and lesions within 8 days (Text-fig. 2, *b* and *c*). The leptospiras were demonstrated in the blood and emulsions of the liver and kidneys. Pure cultures were obtained from the blood of these guinea pigs.

The seventh positive transmission was obtained with a culture derived directly from the blood of Patient A. Ce.

Case 6 (*Text-Fig. 3, a*).—A. Ce., female, age 18 years; seamstress, from a coast town of Vinces. Had been in Guayaquil 12 days.

Onset with chills and fever on Oct. 16, 1918, followed by headache, pains in the back, legs, and epigastric region. Vomited yellowish matter. Admitted on Oct. 18. Slight jaundice; marked albuminuria; intense pain throughout the body; nausea. Several petechial hemorrhages on the chest and arms. Oct. 19. Jaundice more distinct; tongue coated, with free edge and tip; bilious vomit; albuminuria; oliguria. Patient gravely ill. Blood culture made. Oct. 20. All symptoms becoming worse. Black vomit and profuse bleeding from the gums. Oct. 21. Comatose all day. Oct. 22, 8 a.m. Died. Autopsy not performed.

The culture tubes were examined on October 26, 1918, 7 days after they were set up, by the dark-field microscope. Only one of the six tubes showed the presence of active leptospiras in small numbers. From this twelve subcultures were made. The other tubes remained apparently without growth. Inoculations into guinea pigs were made at three different laboratories, first in the laboratory of the Yellow Fever Hospital in Guayaquil on October 27, then in the laboratory of the Colon Hospital, Panama Canal, on November 7, and finally in the laboratory of The Rockefeller Institute on December 11 and 19; that is, 8, 17, 44, and 52 days respectively after the time of making the cultures. Subsequent subcultures



TEXT-FIG. 3, *a* to *d*. Indirect transmission from patient to guinea pig by means of culture derived from the blood of Patient Ce., Case 6. (*a*) Case 6. Age 18 years. (*b*) Guinea pig. Strain, Case 6. Second passage from culture. (*c*) Guinea pig. Strain, Case 6. Culture 2. (*d*) Guinea pig. Strain, Case 6. Culture 3.

of this strain have since been obtained (Figs. 5 and 6). The protocols of some of these series of inoculations of guinea pigs with the cultures follow.

Animal Inoculation (*Second Series*). *Guinea Pig CeI*$_2$ (*Text-Fig. 3, c*).—Nov. 7, 1918. Inoculated intraperitoneally with 0.5 cc. of the culture (17 days old) of blood from Patient Ce. Temperature 39.8°C. on the 4th day, 40° on the 5th, 39° on the 6th, and 37.2° on the 7th. Suffusion of the conjunctivæ, ears, and soles. Slight jaundice appeared on the scleras, ears, and skin on the 7th day. The urine contained bile pigment and albumin. The animal was killed on Nov. 13 for examination and culture.

Autopsy.—Moderate general jaundice. Many spots of hemorrhage in lungs; liver congested and brownish dark red; kidneys swollen and cloudy; gastrointestinal mucosa hyperemic and somewhat hemorrhagic; spleen not altered.

Guinea Pig CeI$_3$ (*Text-Fig. 3, d*).—The same as the foregoing experiment. The animal showed symptoms and lesions almost identical with those just described.

Animal Inoculation (*Third Series*). *Guinea Pig 4, Second Passage from No. CeI*$_2$ (*Text-Fig. 3, b*).—Dec. 11, 1918. Inoculated intraperitoneally with 0.5 cc. of the liver emulsion of guinea pig infected with the first generation of culture of Ce. strain (35 days old) which had never been passed through any animal. Temperature 39.5°C. on the 4th, 39.8° on the 5th, 40.6° on the 6th, 38.8° on the 7th, 41° on the 8th, 40° on the 9th, 38° on the 10th, and 37.6° on the 11th day. The animal became intensely yellow throughout the entire body and was killed for culture and examination.

Autopsy.—All tissues were deeply jaundiced, and there were some minute ecchymoses in the subcutaneous and muscular tissues. The lungs showed large and small irregular hemorrhagic spots which on section were seen to extend into the parenchyma. The color of the liver was strikingly yellow. Kidneys swollen and on section cloudy; marking indistinct. Substance of kidney deeply jaundiced. Suprarenal glands congested. Mucosa of the gastrointestinal tract hyperemic. Some hemorrhages, staining the contents blackish red. Spleen not visibly affected. Only a few leptospiras found in the kidneys and none in the blood or liver.

In addition to the animals given above five more guinea pigs were inoculated in the same series. All showed symptoms and lesions similar to those just outlined. Fig. 4 shows the organism in a stained blood preparation from one of the infected guinea pigs.

The instance just described confirms the results obtained in the first positive transmission experiment (Case A.); namely, that the leptospira can be directly cultivated from the blood of yellow fever patients and then inoculated into guinea pigs with reproduction of

the symptoms and lesions which characterize the disease in man. As we shall note again elsewhere, the virulence of this organism was maintained in cultivation for more than 5 weeks.

To sum up the results recorded in the preceding series of experiments, it is shown that there exists in certain cases of yellow fever prevalent in Guayaquil a definite organism which is capable of being transmitted to the guinea pig and reproduces the symptom complex characteristic of yellow fever. As other experiments showed, the organism which has been observed and isolated in Guayaquil is a leptospira (Figs. 1 to 6) closely resembling the *Leptospira icterohæmorrhagiæ* of Inada and Ido,[10] discovered by them in the infectious jaundice of temperate climates. That the present organism is closely allied to but immunologically distinct from that species of the infectious jaundice has since been established and the experiments bearing on this point will be discussed in detail in subsequent papers. For the present I shall refer to the organism isolated from the yellow fever (typhus icteroides) cases as the yellow fever leptospira, or *Leptospira icteroides* (ἴκτερος, jaundice, + εἰδης, resembling, like), thus denoting the source from which it was obtained.

Negative and Abortive Infections.

In the twenty-one instances to be recorded there was either a temporary febrile reaction in the guinea pigs after inoculation of the blood, with or without any suspicion of jaundice, or almost no reaction at all. In other words, the results are classed as indefinite or negative. It is noteworthy, however, that a considerable proportion of the guinea pigs inoculated with the blood drawn during the first 5 days of the disease had a febrile reaction either on the 4th, 5th, 6th, or 7th day, and in some of these animals even a trace of jaundice was noted or suspected for a day or two soon after the fever. In none did the jaundice become definite, and the animals subsequently returned to normal. Undoubtedly, by transferring the virus to normal animals at this stage more strains might have been secured in passage, but much time was lost in watching further development of the symptoms, and opportunities to obtain the strains were lost. Some of these animals must have had a mild or abortive

TABLE I.

Case No.	Sex.	Age.	Course of disease.	Day of disease on which blood was taken.	No. of guinea pigs inoculated.	Outcome of inoculation.
		yrs.				
7	Male.	21	Severe; recovery.	2nd	2	Febrile reaction only.
8	"	21	" "	2nd	2	" " in one, fever and trace of icterus in other; recovery in both.
9	"	19	Moderate; "	2nd	2	No reaction in one, fever and trace of icterus in other; recovery in both.
10	Female.	26	Severe; died on 7th day.	2nd	2	No reaction.
11	"	10	Extremely mild.	2nd	2	" " in one, slight fever in other.
12	"	40	Moderate; recovery.	2nd	2	Febrile reaction in both.
13	Male.	35	Mild; "	3rd	2	" " " "
14	"	23	Severe; "	3rd	2	No reaction in one, fever in other.
15	"	25	Very mild; "	3rd	2	Fever in both.
16	"	25	Moderate; "	3rd	2	" " " and suspicion of icterus; both recovered.
17	"	19	Severe; died on 6th day.	3rd	1	Fever and suspected trace of icterus; recovered.
18	"	23	Severe; died on 7th day.	4th	1	Fever and suspicious yellow tint in scleras; recovered.
19	"	25	Severe; recovery.	4th	2	Febrile reaction in both and suspicion of icterus in one.
20	"	20	" "	4th	2	Fever in both and perhaps a trace of icterus.
21	"	28	Severe; died on 4th day.	4th	3	All had a febrile reaction but no icterus.
22	"	16	Severe; died on 10th day.	5th	2	Both had fever and suspiciously yellow scleras.
23	"	16	Moderate; recovery.	5th	1	Febrile reaction.
24	"	20	Severe; died in 6 (?) days.	5th (?)	2	Both had fever. Suspicion of icterus in one.
25	Female.	21	Mild; recovery.	6th	2	Fever in one, no reaction in other.
26	Male.	21	Severe; "	8th	2	No reaction in either.
27	"	32	" died on 16th day.	14th	2	" " " "
27	"	32	Severe; died on 16th day.	15th	2	" " " "

form of the infection, as they subsequently proved to be refractory to a virulent virus when tested after a period of about 25 days from the time of the inoculation of the yellow fever blood. For the sake of completeness the protocols of the negative transmission experiments are recorded in Table I.

SUMMARY.

By injecting into guinea pigs the blood of yellow fever cases occurring in Guayaquil a group of symptoms and lesions closely resembling those observed in human yellow fever were induced in a limited number of instances. Of 74 guinea pigs inoculated with specimens of blood from 27 cases of yellow fever, 8, representing 6 cases, came down with the symptoms; namely, a marked rise of temperature after a period of incubation averaging 3 to 6 days, with simultaneous suffusion of the capillaries, particularly of the conjunctivæ and soles, then preliminary hyperleucocytosis followed by progressive leucopenia, the early appearance of albumin and casts in the urine, which gradually diminishes in volume as the disease progresses. The fever lasts only a few days, rapidly dropping first to the normal and then usually to subnormal. At this period jaundice manifests itself in varying degrees of intensity, first in the scleras, then in the skin and the urine. Hemorrhages from the nasal or gingival mucosa or anus have been observed to occur during this period. Autopsies reveal deep jaundice throughout the entire tissue. The liver is fatty and yellow, the kidney hyperemic, and often swollen and hemorrhagic. Hemorrhagic spots were almost always found in the lungs and gastrointestinal mucosa. Guinea pigs are usually rather sensitive to the infection, though many appeared to be somewhat resistant and some even refractory.

The injection of the yellow fever blood into ringtail monkeys, rabbits, cats, guatusas, weasels, and sloths among the mammalians, and pigeons, ground-doves, bluebirds, mantas, blackbirds, parrakeets, reedbirds, blancos, and toucans among the birds, gave negative results.

In the blood, liver, and kidneys of the guinea pigs experimentally infected with the blood of yellow fever patients a minute organism

was demonstrated which closely resembles in morphology the causative agent of infectious jaundice (*Leptospira icterohæmorrhagiæ*).

The leptospira transmitted from yellow fever cases to guinea pigs was found to induce similar symptoms and lesions upon further passage into normal guinea pigs.

The leptospira obtained from cases of yellow fever has been given the provisional name of *Leptospira icteroides*.

I wish to express my thanks to Dr. León Becerra, General Director of the Department of Health of Ecuador, and his staff for their support and cooperation in the execution of this work; and likewise to Dr. Pareja of the Guayaquil Yellow Fever Hospital and to his laboratory staff (Dr. Larrea), clinical staff (Dr. Davila and Dr. Martinez), and nursing staff for their invaluable assistance.

To Dr. Herman B. Parker I am indebted for his courtesy in lending me a dark-field arc lamp during the period before my own arrived, when the dark-field work would otherwise have been impossible, and also in furnishing me on various occasions with clinical material.

I am indebted to Colonel McCormack, Chief Health Officer of the Canal Zone, to Major Teague and Captain McFarland of the Ancon Hospital, and to Captain Bowen and Lieutenant Levy of the Colon Hospital, for the facilities afforded me in making renewal of cultures and transfers of strains to new animals.

EXPLANATION OF PLATE 35.

FIG. 1. *Leptospira icteroides* in the blood of a guinea pig experimentally inoculated with culture of strain from Patient A. A., Case 1. Fixed in methyl alcohol and stained with Wright's stain. The film was made on the 6th day of illness. × 1,000.

FIG. 2. The same; strain from Patient M. G., Case 3. × 1,000.

FIG. 3. The same; strain from Patient Co., Case 4. × 1,000.

FIG. 4. The same; strain from Patient A. Ce., Case 6. × 1,000.

FIG. 5. Dark-field view of a culture 16 days old of *Leptospira icteroides*. Strain from Patient A. Ce., Case 6. × 1,000.

FIG. 6. The same. × 1,000.

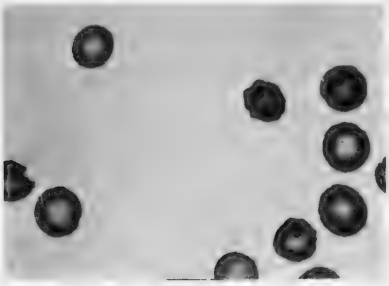


FIG. 1.

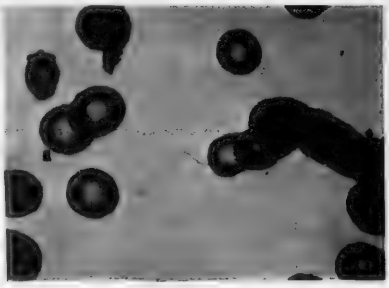


FIG. 2.

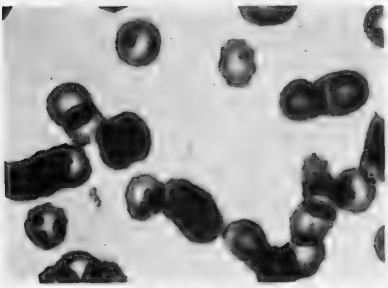


FIG. 3.

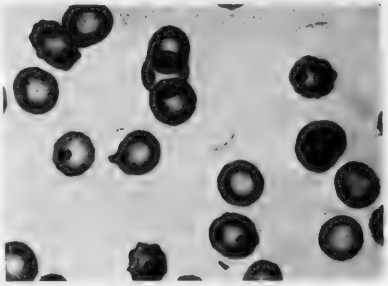


FIG. 4.

FIG. 5.

FIG. 6.

(Noguchi: Etiology of yellow fever. II.)

ETIOLOGY OF YELLOW FEVER.

III. Symptomatology and Pathological Findings in Animals Experimentally Infected.

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Plates 36 to 38.

(Received for publication, March 26, 1919.)

Mention has already been made[1] of successfully inducing in guinea pigs symptoms and pathological conditions simulating those observed in yellow fever patients in Guayaquil by inoculating these animals with the blood or organ emulsions from yellow fever patients. It has also been stated[1] that in the blood and organ emulsions of the infected guinea pigs an organism belonging to the genus *Leptospira* has been demonstrated and that the organism, after having been obtained in culture, is capable of inducing the same symptoms and pathological changes in these animals as does the original blood of yellow fever patients. In this paper the mode and course of this infection as observed in guinea pigs, dogs, and monkeys will be described.

Mode of Experimental Infection.

Infection with this organism may be induced either by injection into the peritoneal cavity, the blood circulation, or the subcutaneous tissues, or by application to the scarified, depilated surface of the skin or to the mucous membranes, or by feeding the animal with infected tissue or culture.

Experimental Infection in Guinea Pigs (Text-Fig. 1, a to f).

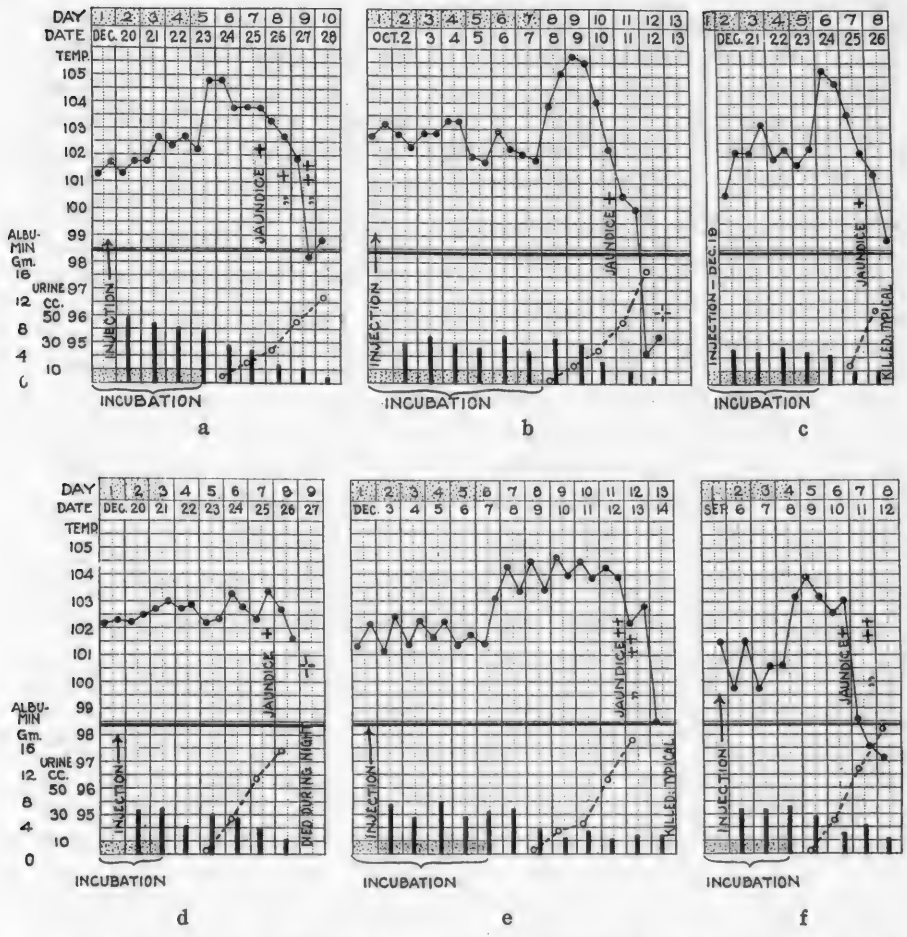
Incubation Period.—The incubation period varies according to the mode of infection and the quantity of virus introduced. When a large

[1] Noguchi, H., Etiology of yellow fever. II. Transmission experiments on yellow fever, *J. Exp. Med.*, 1919, xxix, 565.

amount is inoculated intraperitoneally or into the circulation the first symptoms—inactivity, anorexia, hyperleucocytosis—make their appearance after 48 hours, followed by a rise in temperature and slight albuminuria within the next 24 hours. With subcutaneous inoculation the symptoms do not appear until the 4th (72 hours) or 5th (96 hours) day, and with a very small amount of the virus a few days later. The percutaneous and *per os* modes of infection require a period of about 6 and sometimes as many as 12 days of incubation.

Onset and Course.—The onset is indicated by loss of appetite, inactivity, injection of the bulbar conjunctivæ, and a sudden rise in temperature ranging from 39.8–40.5°C. and rarely 41°C. The animal offers little resistance to handling, plaintive cries indicating intense muscular pains. The urine diminishes in quantity from an average daily output of about 25 cc. to almost half that, and its color changes from a pale straw tinge to dark brownish yellow. It now contains a moderate amount of albumin, with some epithelial cells and granular casts. At this stage no bile pigment can be demonstrated in the blood serum and no jaundice in the scleras or other parts of the body. The leucocytes are increased during the 1st day, but leucopenia follows within a few days, the differential count showing a marked increase of polymorphonuclear leucocytes.[2] Within the next 24 hours the temperature shows a slight drop, only to return to 39.8–40°C. during the following day. The fever then subsides gradually until the temperature drops below the normal in 3 or 4 days more. In fatal cases it sometimes drops to 34°C. just before death. Although the temperature begins to fall on the 2nd day all the other symptoms are aggravated. The urine is reduced still further in quantity and contains an enormous amount of albumin, renal epithelia, granular and hyaline casts, and the bile pigment first appears. At the same time the scleras become deeper yellow and are intensely suffused. At this stage there is a trace of bile pigment in the serum. On the 3rd day icterus becomes quite distinct

[2] I am greatly indebted to Dr. Jorge Larrea, Director of the Laboratories of the Guayaquil Yellow Fever Hospital, for total leucocyte counts on some of the experimental animals. My thanks are also due to Dr. F. Rojas of the General Hospital in Guayaquil.

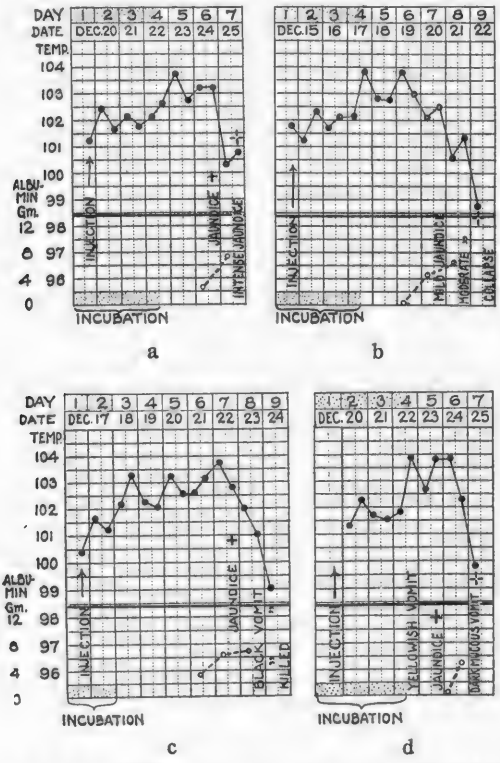


TEXT-FIG. 1, *a* to *f*. Observations on guinea pigs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Guinea pig. Strain, Case 5. (*b*) Guinea pig. Strain, Case 5. (*c*) Guinea pig. Strain, Case 6. (*d*) Guinea pig. Strain, Case 5. (*e*) Guinea pig. Strain, Case 6. (*f*) Guinea pig. Strain, Case 1.

in the ears, scleras, palms, and soles. There may be ecchymoses in the scleras. Epistaxis and hematuria are frequent symptoms. The urine is now a deep grayish brown hue, and only a few cubic centimeters may be excreted. The animal still keeps on its feet but is quiet. On the 4th day the jaundice becomes general, and the serum taken is deep brownish yellow, as is also the urine. Erythrocytes, renal cells, albumin, and bile pigment are very abundant in the scanty urine which may still be passed, or there may be total anuria. Melena or bleeding from the rectum has been observed, and epistaxis is a frequent phenomenon in a dying animal (Fig. 13). Death occurs between the 5th and 7th days after onset of the disease and is accompanied either by clonic convulsions or by gradual asphyxiation, with air-hunger. The blood pressure becomes so low that about 2 days before death very little or almost no blood flows from the largest ear veins, even when they are completely cut across.

Non-Fatal and Abortive Infections.—Experimental yellow fever in the guinea pig is not always fatal. A certain proportion of the animals inoculated with a virulent strain of the virus show a temporary febrile reaction and albuminuria with few casts. Jaundice is slightly noticeable in the ears (if the animal is white or light in color) and scleras, but it is sometimes apparently absent. The fever and other symptoms set in as in fatal cases but completely pass away within a few days. In a week the animal has regained its previous health. That these reactions are mild or atypical symptoms of the same infection is evident from the fact that such animals cannot be reinfected, even with a quantity of the virus sufficient to kill control guinea pigs with the typical symptoms and lesions. Furthermore, these mild infections occur among guinea pigs inoculated with the same quantity of virus which produces a fatal infection among others in the same group, and especially do they occur frequently when the amount of the virus injected is rather small or the strain attenuated in virulence for the guinea pig. As in yellow fever in man, therefore, so in the experimental condition in guinea pigs, there apparently exist varying grades of severity of infection according to the individual resistance to the same virus. Many instances have been encountered, among many hundreds of guinea pigs purchased in Guayaquil, in which the animals showed a complete immunity to the

inoculation of the virus. It is possible that as some of them had been kept in houses or markets for varying periods of time before purchase they were rendered immune through a previous mild infection.



TEXT-FIG. 2, *a* to *d*. Observations on dogs inoculated with the cultures of the different strains of *Leptospira icteroides*. (*a*) Dog (pup). Strain, Case 6. (*b*) Dog (pup). Strain, Case 1. (*c*) Dog (pup). Strain, Case 5. (*d*) Dog (pup). Strain, Case 3.

Experimental Infection in Dogs (Text-Fig. 2, a to d).

Incubation Period.—This is practically the same as in guinea pigs.

Onset and Course.—The onset and course are also similar to those observed in guinea pigs, some dogs succumbing to the infection and

others recovering from a mild attack. In a fatal infection the temperature may reach 40°C., but usually it is not over 39.5°C., and lasts for 3 or 4 days, when it gradually drops by lysis. The skin then becomes yellowish and the conjunctivæ injected, and the animal refuses to eat. It often vomits bilious and sometimes blackish, mucous, frothy contents from the stomach. The amount of urine diminishes, with increasing albumin, casts, and bile pigments. Death occurs in coma or in clonic convulsions, the temperature falling below normal.

Experimental Infection in Monkeys (Text-Fig. 3, a to e).

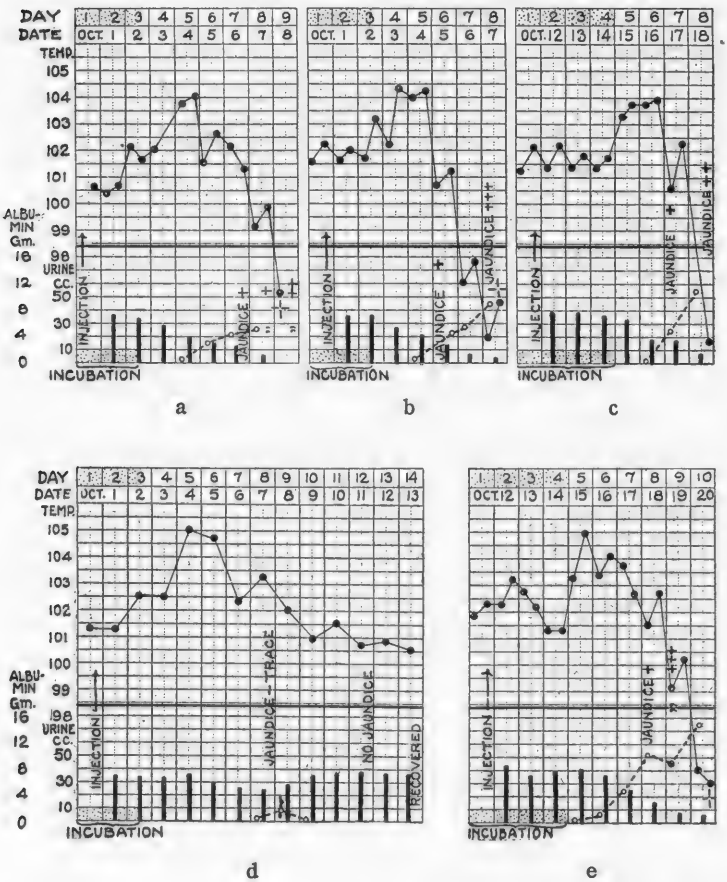
As stated elsewhere, the South American ringtail monkeys were found refractory to infection with the blood of yellow fever patients or the culture of the yellow fever leptospira.[1] With both a temporary febrile reaction was observed, but the animal invariably recovered. Five marmosets,[3] however, proved to be susceptible to the organism, four being fatally and one mildly infected.

The incubation period varied from 3 to 4 days, during which time the animals appeared well. The disease became noticeable by their inactivity, loss of appetite, non-resistance to handling, and slight greenish pigmentation of the urine. Albumin, casts, and bile pigments began to appear in the urine when the fever reached its height. Mild jaundice in the scleras, abdominal skin, and oral mucous membrane became noticeable a day or two after the fever began to subside. On the day of death the animals lay in a helpless position, offering only feeble resistance to handling. Death occurred during a condition of general weakness, coma, and sometimes convulsions. The temperature was usually subnormal, and the body of the animal more distinctly and generally yellowish.

Autopsy Findings in the Experimental Infection (Fig. 14).

The guinea pigs, dogs, and marmosets dying of the experimental infection invariably show a pronounced jaundice ranging from a light yellow to a deep bright yellow. Blood clots or stains are often

[3] Species *Midas œdipus* and *Midas geoffroyi.*



TEXT-FIG. 3, *a* to *e*. Observations on monkeys inoculated with the different strains of *Leptospira icteroides*. (*a*) Monkey 1. Strain, Case 1. Culture of second generation, 2 cc. intraperitoneally. (*b*) Monkey 2. Strain, Case 3. Culture of first generation, 4 cc. intraperitoneally. (*c*) Monkey 5. Strain, Case 6. Guinea pig kidney emulsion, 6 cc. intraperitoneally. (*d*) Monkey 3. Strain, Case 1. Culture of second generation, 4 cc. intraperitoneally. (*e*) Monkey 4. Strain, Case 5. Guinea pig liver emulsion, 6 cc. intraperitoneally.

present in the nostrils, mouth, anus, or vagina. Subcutaneous ecchymoses are frequently present in the guinea pig but seldom in the dog or marmoset. Postmortem rigor and lividity are marked. In guinea pigs the subcutaneous tissues are intensely jaundiced, and ecchymoses are seen in the axillary and inguinal regions. The abdominal muscles are often spotted with minute ecchymoses but sometimes these are absent. In the dog and marmoset the muscles show very slight or no hemorrhage.

The lungs of the guinea pig almost always show ecchymoses varying in extent from few and minute to numerous and large ones, roughly round, oval, or irregularly oblong or square, and with or without a sharp outline, vivid red, dark, or bluish red in color, sometimes light red with a darker center. Undoubtedly the color of the spots becomes darker and more bluish as they become older, since the longer the animal lives the darker are these ecchymoses. Postmortem hypostasis is marked. There may be some ecchymoses in the pleuræ. In the dog and marmoset the lungs are much less affected, only a few spots of hemorrhage being found, and the pleuræ are usually free.

There may be some dilation of the right heart. The pericardium is often studded with minute hemorrhagic spots. The fluid is clear and icteric. The muscle is friable and brownish yellow, and there are few or sometimes many ecchymoses on the surface. The endocardium and papillary muscles seem normal except for occasional punctiform ecchymoses. The valves are not altered but are yellowish in color, the aorta usually being deeply jaundiced. These changes apply equally to the guinea pig, dog, and marmoset.

The liver is usually slightly enlarged and varies in color from a yellowish brown-red to a bright yellowish brown. In instances in which death occurred within the first 3 days the degeneration was less advanced and the yellowish color not so pronounced. The surface is often mottled or striped with yellowish brown and brownish red, and the markings are very distinct.

The gall bladder is usually filled with a dark green or greenish yellow bile, and the wall is often spotted with minute ecchymoses.

The stomach usually contains some undigested food which is mixed with blood from the adjacent ecchymotic area of the mucosa. The contents are sometimes semifluid containing blackish blood re-

sembling coffee-grounds in appearance. In dogs and marmosets the greater quantity of mucus renders the appearance of the stomach contents indistinguishable from those found in human autopsies. The mucosa is somewhat hyperemic, and numerous ecchymoses are found, especially near the cardia. The serosa of dogs and marmosets is free of hemorrhages.

The small intestine and colon, including the rectum, are intensely injected, and numerous hemorrhages are found in the mucosa. The serosa is sometimes affected. The contents are blackish in color and may contain freshly escaped blood (melena). In the dog the character of the intestinal contents is a closer reproduction of what is observed in man, the serosa in this animal not showing the ecchymoses which are present in the guinea pig or any appreciable fluid in the peritoneal cavity. The nature of the findings in marmosets is about midway between that of guinea pigs and that of dogs.

In guinea pigs the kidneys are extensively involved. Hyperemia and punctiform hemorrhages in the parenchyma are almost constant. The ecchymoses, however, vary from a few to almost countless numbers in extreme instances. In dogs and marmosets there may be only a few minute spots. On section the cortex is broader than normally and highly hyperemic, with cloudy swelling. The medullary portion is succulent and hyperemic near the border. Bloody fluid or clot may be found in the pelvis, and sometimes numerous punctiform ecchymoses.

In the guinea pig hyperemia and hemorrhages were found in the suprarenal glands, but in dogs and marmosets only a comparatively slight degree of hyperemia. There were no changes in the pancreas or spleen, except a slight enlargement of the latter in rare instances.

The lymphatic glands show, in guinea pigs, general adenopathy, with occasional hemorrhages. In dogs and marmosets some glands only are enlarged and congested.

The bladder frequently contains bile-stained urine full of albumin, casts, and cells.

The testicles are apparently not affected, although in the guinea pig hemorrhages are frequent in the adipose tissue around them.

The ovaries are usually congested, and the uterus very much so, and in the pregnant state there were hemorrhages into the amnionic

fluid. The endometrium is congested, sometimes with a clot in the cavity.

No gross changes are observed in the nervous system. The membranes are hyperemic and the fluid is icteric.

Histological Examination (*Figs. 1 to 12*).

Lungs (*Figs. 3 and 6*).—The areas of hemorrhage are most abundant and extensive in the guinea pig. The alveoli in the hemorrhagic areas are completely filled with blood corpuscles, and in the adjacent zone a marked degree of edema is evident. There are also small accumulations of polymorphonuclear leucocytes and endothelial cells. In marmosets and dogs these changes are far less extensive. The leptospiras were demonstrated in the tissues by the Levaditi method.

Liver (*Figs. 1 and 4*).—The degree of degeneration of the liver cells is variable in different animals. In well marked instances the majority of the cells are swollen and necrotic. Vacuoles are found in some cells. The nuclei are swollen and degenerated. In some areas the liver cells are dissociated, swollen, and appear to have lost their sharp hexagonal outlines. The liver cells near the blood vessels seem to be less affected than those around the portal canal. Hemorrhagic foci of varying dimension are distributed irregularly. The endothelial cells of the bile ducts are increased in size, and there are some lymphoid cells around the portal canal zone. Mitotic figures are found. The organisms were found in the tissues stained by Noguchi's method (Figs. 7 and 10).

Kidneys (*Figs. 2 and 5*).—The epithelium of the convoluted tubules shows granular, swollen, and sometimes vacuolated cytoplasm. The cells may be detached from the membrane and fill up the lumen, which is distended with granular and hyaline casts. The glomeruli are considerably injected, and numerous hemorrhages are found throughout the cortex and medulla (Figs. 8, 9, 11 and 12).

Stomach.—There are superficial congestion of the mucosa and some hemorrhagic foci. In certain areas there is an accumulation of lymphoid and plasma cells.

Large and Small Intestines.—The intestines show injection and occasional hemorrhages.

Heart.—Certain fibers are swollen and contain vesicular nuclei.

Spleen.—There are hemorrhages, and the pulp is rich in blood.

Lymph Nodes.—These show phagocytosis and central degeneration of the follicles.

Adrenal.—In guinea pigs there are parenchymatous degeneration, congestion, and hemorrhages in some instances. These changes are less frequent in dogs and marmosets.

Pancreas.—Little change.

Nervous System.—Little change.

SUMMARY.

Studies are reported on the type of disease induced in guinea pigs, dogs, and monkeys by inoculating them (1) with the blood or organ emulsions of guinea pigs or other susceptible animals experimentally infected with *Leptospira icteroides*, and (2) with a pure culture of the organism. Particular attention has been given in these experiments to the clinical features of the experimental infection in the various animals and to the pathological changes resulting from the infection.

The symptoms and pathological lesions induced in guinea pigs are much more pronounced than those observed in dogs or marmosets. The period of incubation is nearly the same in all three species, 72 to 96 hours with intraperitoneal or subcutaneous inoculation, and a day or more longer when the infection is induced percutaneously or *per os*. The febrile reaction in the guinea pig and marmoset is about the same; in the dog there is less fever. The amount of albumin, casts, and bile pigments in the urine is more abundant in the guinea pig and marmoset than in the dog, and these animals also appear on the whole to become more intensely icteric. The black or bilious vomit, however, though occurring frequently in dogs during life, is observed in the guinea pig and marmoset at autopsy. The hemorrhagic diathesis is most pronounced in guinea pigs, less so in marmosets, and least in dogs. In dogs, for example, subcutaneous hemorrhages almost never occur, and the lungs usually show only a few minute ecchymoses. The pleuræ, pericardium, and other serous surfaces of the thorax and abdomen remain free from ecchymoses,

which, however, with hyperemia, are very marked along the gastro-intestinal tract.

The symptoms and lesions observed in animals experimentally infected with *Leptospira icteroides* closely parallel those of human yellow fever.

The pathological changes occurring in human cases of yellow fever are similar to those induced by inoculation in guinea pigs and marmosets and in respect to their intensity stand intermediate between those arising in the two animals mentioned.

EXPLANATION OF PLATES.

PLATE 36.

Sections of tissues from yellow fever cases, fixed in Zenker's fluid and stained with eosin and methylene blue. × 150.

FIG. 1. Section of the liver of a marmoset inoculated with the organism isolated from Patient E. Ch., Case 5, showing hemorrhage and necrosis, vacuolization, and dissociation of the liver cells.

FIG. 2. Section of the kidney from the same marmoset, showing necrosis, detachment, and some vacuolization of the renal epithelia. Some of the lumina are seen to contain granular casts. The glomeruli seem to be highly congested; hemorrhage in some portions of the tissue.

FIG. 3. Section of the lung from the same marmoset, showing hemorrhage in the tissue.

FIG. 4. Section of the liver from a guinea pig inoculated with the organism isolated from Patient M. G., Case 3. The liver cells are seen to have been largely disintegrated and replaced by red corpuscles and debris.

FIG. 5. Section from the kidney of same guinea pig, showing advanced degeneration of renal epithelia, with granular and hyaline casts in the lumen.

FIG. 6. Section of the lung from a guinea pig inoculated with the strain isolated from Patient A. Ce., Case 7. There is a considerable degree of hemorrhage and edema. Some leucocytic infiltration seems to be evident.

PLATE 37.

Sections from the liver and kidney of marmosets and guinea pigs inoculated with the strains of *Leptospira icteroides* derived from Cases 1, 3, and 5. Figs. 10 to 12 are stained by the method of Levaditi, Figs. 7 to 9 by the writer's modification of that method. × 1,000.

FIG. 7. Liver of marmoset, showing the organism between the hepatic cells; strain from Case 5.

FIG. 8. The same strain. There are two minute, delicate organisms in the lumen of a kidney tubule.

FIG. 9. The same.

FIG. 10. The organism in the guinea pig liver, in which it appears somewhat coarse.

FIGS. 11 and 12. The organism in the kidney; coarse and quite numerous.

PLATE 38.

FIG. 13. Epistaxis in a guinea pig inoculated with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The picture was taken at the moment of death.

FIG. 14. Autopsy of a guinea pig killed on the 6th day after inoculation with a culture of *Leptospira icteroides* isolated from Patient A. Ce., Case 6. The animal showed typical symptoms and was killed at the time of collapse. The picture shows the general jaundice of the skin and subcutaneous tissues and the yellowish liver. Hemorrhages are evident in the lungs, and can be seen to have taken place in the stomach, as shown by its dark color. The kidney is congested, and sometimes a few ecchymotic spots can be seen. The spleen appears to be normal. The intestines and also the muscles along the thorax, abdomen, and postperitoneal region are comparatively free from hemorrhage, presenting a striking contrast to the highly congested condition of these muscles in guinea pigs inoculated with the organism of infectious jaundice. The strikingly yellow color of the liver is far more intense than is usually observed in experimental infectious jaundice in the guinea pig.

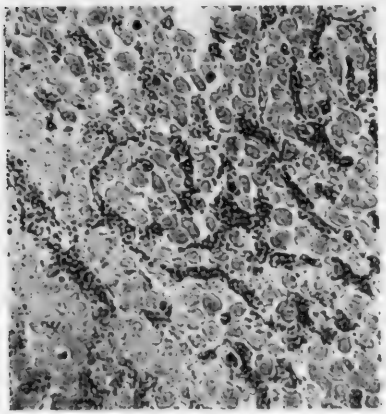
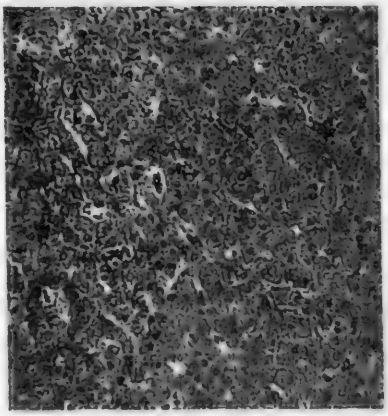


FIG. 1.



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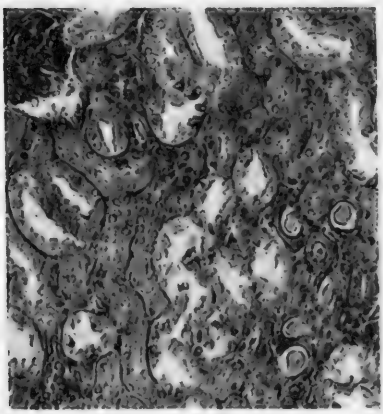
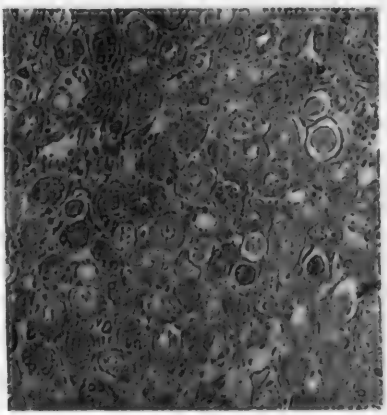


FIG. 2.



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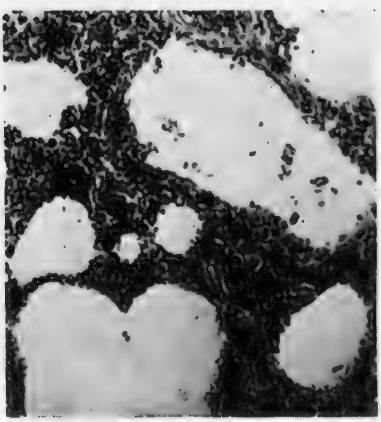

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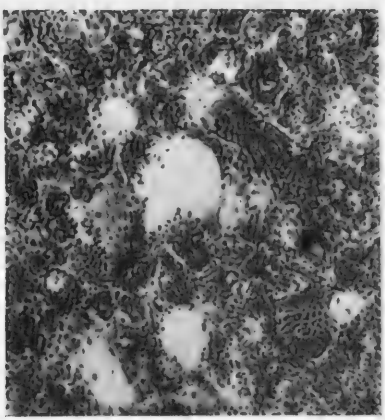


FIG. 6.

(Noguchi: Etiology of yellow fever. III.)

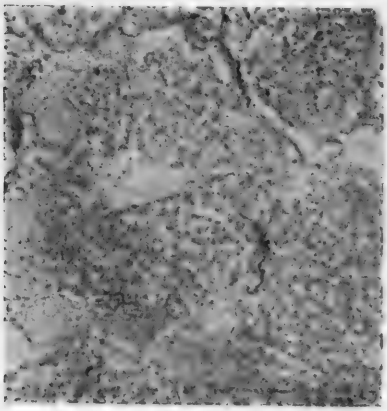


FIG. 7.

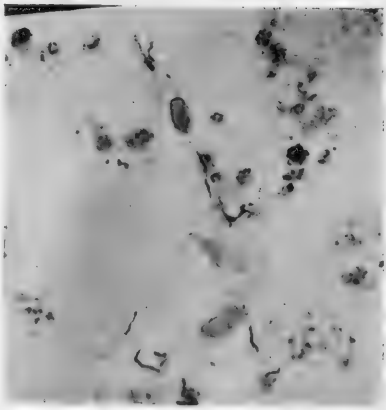
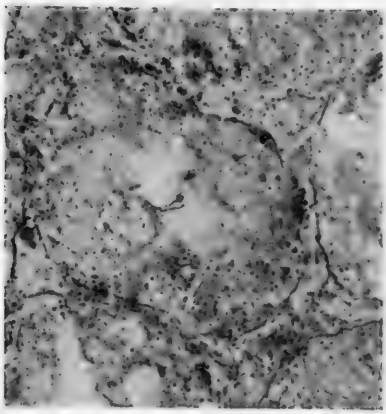


FIG. 10.



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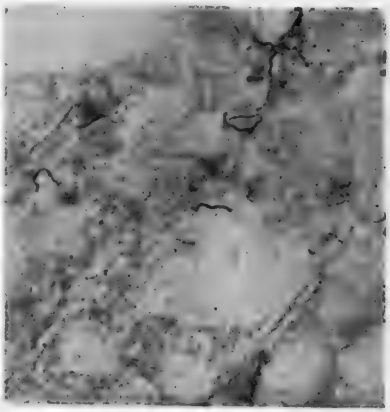


FIG. 11.

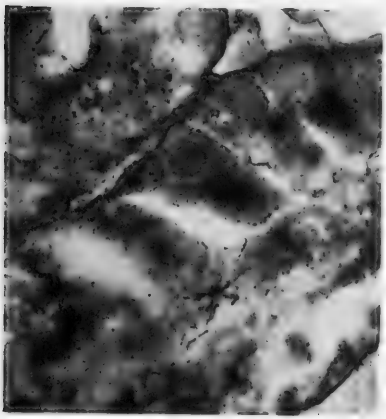
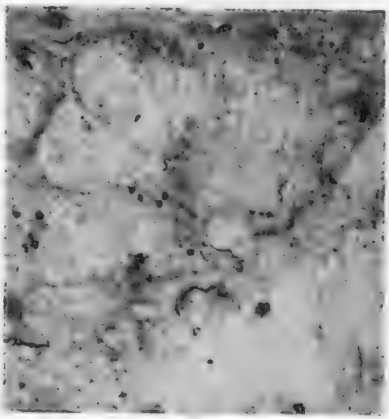


FIG. 9.



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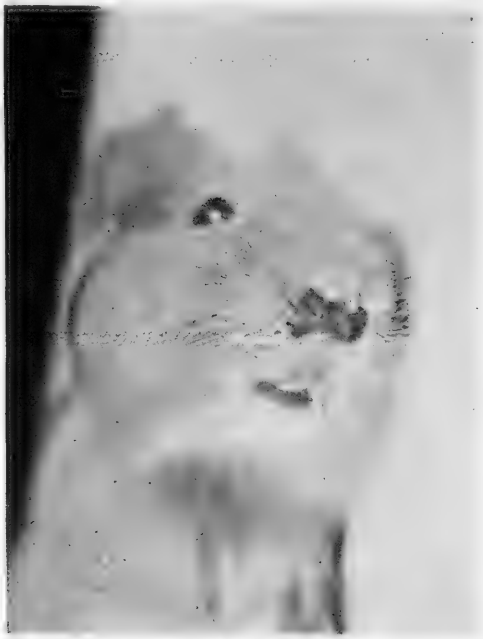

FIG. 13.

FIG. 14.

THE PASSAGE OF MENINGOCOCCIC AGGLUTININS FROM THE BLOOD TO THE SPINAL FLUID OF THE MONKEY.

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The administration of antimeningococcic serum for the treatment of epidemic meningitis by other than the intraspinal route has hardly been considered until recently, since the publication of the papers by Flexner and his coworkers[1,2] on the specific serum therapy of the disease on which the prevailing mode of treatment is based. The experience with a large number of cases of epidemic meningitis during the great war and under the unusual conditions of camp life has led to the revival of the employment of the serum by intravenous, usually associated with intraspinal, injection. That the intravenous administration was both indicated and called for is evidenced by the occurrence of cases of meningococcemia in some instances without, and in others before the onset of, the meningitis.[3,4] Although other clinicians have from time to time employed antimeningococcic serum by intravenous injection the systematic use of it in that manner has been developed especially by Herrick.

The purpose of the intravenous injection may be regarded as threefold: (1) to combat the meningococcemia; (2) to diminish possibly the rapidity of the passage of the serum from the subarachnoid space into the blood; and (3) to bring remote portions of the meninges, not so readily accessible from the spinal fluid itself, under the influ-

[1] Flexner, S., *J. Am. Med. Assn.*, 1906, xlvii, 560; *J. Exp. Med.*, 1917, ix, 168; 1913, xvii, 553.

[2] Flexner, S., and Jobling, J. W., *J. Exp. Med.*, 1908, x, 141, 690.

[3] Herrick, W. W., *J. Am. Med. Assn.*, 1918, lxxi, 62; *Arch. Int. Med.*, 1918, xxi, 541.

[4] Baeslack, F. W., Bunce, A. H., Brunelle, G. C., Fleming, J. S., Klugh, G. F., McLean, E. H., and Salomon, A. V., *J. Am. Med. Assn.*, 1918, lxx, 684.

ence of the serum. Under normal conditions antibodies do not pass from the blood into the cerebrospinal fluid,[5] but under circumstances of inflammation of the meninges the permeability is increased, and passage may in some degree take place. Of the three possibilities, the first, namely the control of the blood invasion by meningococci, may be regarded as the most important, since it may, if only in rare instances, prevent or abort a meningeal infection, and it may be even more effective in preventing the infections of joints, heart, eye, etc.

The experiments to be described relate to the question of the possibility and the degree of passage of antibodies, in this instance agglutinins, for the meningococcus from the blood into the cerebrospinal fluid.

EXPERIMENTAL.

Macacus rhesus monkeys were employed for the experiments because of the ease with which chemical meningitis may be induced in them and especially because they readily yield cerebrospinal fluid on lumbar puncture.

The first experiment was arranged to test two points: (*a*) whether the antimeningococcus agglutinins passed from the blood into the cerebrospinal fluid in normal animals, and (*b*) whether they passed in animals in which a chemical meningitis had been incited when only 10 cc. of the antiserum had been injected into a superficial vein. The protocol which follows shows not only that no such passage takes place in the normal animal but either none that is demonstrable, or at least very little, even in the presence of an aseptic meningitis.

Experiment 1.—Mar. 13, 1918. Three *Macacus rhesus* monkeys, weighing about 3 kilos each, received intravenously 10 cc. of polyvalent antimeningococcic serum. Monkeys A and B had received 18 hours previously 2 cc. of normal horse serum intraspinally. No intraspinal injection was given Monkey C. 7 and 24 hours after the intravenous injection spinal fluid was removed from each and tested for agglutination against the meningococcus. None was observed in either specimen from the monkey which received no intraspinal injection. The same was true of the specimens from one of the monkeys in which chemical meningitis had been induced. The spinal fluid removed 7 hours but not 24 hours after intravenous injection from the second monkey, which had received intraspinal injection of horse serum, agglutinated regular and parameningococcus in a dilution of 1: 2 and 1: 4.

[5] Flexner, S., *J. Am. Med. Assn.*, 1913, lxi, 447, 1872.

The second experiment was made with 20 cc. of polyvalent antimeningococcic serum of high titer which was injected in each instance into a superficial vein. It was devised to cover the following conditions: (*a*) effect on normal cerebrospinal fluid, (*b*) effect on cerebrospinal fluid modified by a mild aseptic meningitis induced by an intraspinal injection of sterile normal salt solution, (*c*) effect on the cerebrospinal fluid modified by a more severe chemical inflammation induced by normal horse serum, and (*d*) a still more severe inflammation brought about by the injection of a salt solution suspension of regular meningococcus culture. In the last instance the agglutinin tested for was that of the para organism.

The result is clear: no agglutinins were present in the cerebrospinal fluid derived from the normal animal which received the intravenous injection; fluctuating amounts were present in the cerebrospinal fluid after salt solution, more after the horse serum injection, and most after the injection of meningococcus. In other words, the normal meninges were not permeable, while the inflamed membranes were permeable in proportion to the intensity of the meningitis experimentally induced.

Experiment 2.—Macacus rhesus D, weight 3.2 kilos; control. Mar. 21, 1918. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. 5.25 p.m. Lumbar puncture, 0.5 cc. of clear fluid which did not agglutinate regular or parameningococcus. Mar. 22, 10.10 a.m. Lumbar puncture, 1 cc. of clear fluid which did not agglutinate the meningococci. 5.50 p.m. Injected intraspinally one-quarter of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 12 m. Lumbar puncture, 0.5 cc. of turbid fluid which, after centrifugation, agglutinated parameningococcus ++ in a dilution of 1:4. Culture from spinal fluid negative. Mar. 24, 10.35 a.m. Lumbar puncture, 0.5 cc. of turbid fluid. Cultures negative. Centrifuged fluid agglutinated parameningococcus + in a dilution of 1:2.

Macacus rhesus E, weight 3.5 kilos. Mar. 20, 1918, 4 p.m. Injected intraspinally 2 cc. of isotonic salt solution. Mar. 21, 11.10 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.30 a.m. Lumbar puncture, 1 cc. of slightly turbid fluid. The centrifuged fluid did not agglutinate regular or parameningococcus in a dilution of 1:2. 5.45 p.m. Injected intraspinally one-quarter of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 11 a.m. Lumbar puncture, 0.5 cc. of turbid fluid which yielded negative culture. The centrifuged fluid agglutinated parameningococcus + in a dilution of 1:4. Mar. 24, 10.25 a.m. Lumbar punc-

ture, 0.5 cc. of slightly turbid fluid which yielded negative culture. The centrifuged fluid agglutinated parameningococcus ++ in a dilution of 1:4.

Macacus rhesus F, weight 3.3 kilos. Mar. 20, 1918, 4 p.m. Injected intraspinally 2 cc. of normal horse serum. Mar. 21, 10.38 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Mar. 22, 10.20 a.m. Lumbar puncture, 0.5 cc. of turbid spinal fluid. The centrifuged fluid agglutinated regular meningococcus ± 1:4, − 1:2, and parameningococcus + 1:4 and − 1:2. 6.15 p.m. Lumbar puncture, 0.5 cc. of turbid fluid which after centrifuging agglutinated parameningococcus + 1:4. Injected intraspinally one-fourth of an 8 hour culture of regular meningococcus in 0.5 cc. of isotonic salt solution. Mar. 23, 11.30 a.m. Lumbar puncture, 0.5 cc. of turbid fluid; culture negative; the centrifuged fluid agglutinated regular meningococcus + in a dilution of 1:2, ++ 1:4, and parameningococcus + 1:2, − 1:4.

The next experiment brings out conclusively the effect of degree of chemical inflammation in promoting the passage of agglutinating bodies from the blood into the spinal fluid. The order of the experiment was first to inject intraspinally into *Macacus rhesus* monkeys either isotonic salt solution or normal horse serum, and about 20 hours later to give 20 cc. of polyvalent antimeningococcic serum intravenously. The spinal fluid is then withdrawn at stated intervals and tested. When salt solution or horse serum is injected intraspinally and the turbid fluid withdrawn later and tested for agglutinins of the meningococcus no agglutination takes place unless antimeningococcic serum has been given also. The results of this experiment are given in Table I. The far greater effect of the horse serum over isotonic salt solution is at once apparent.

Attention has been drawn to the practice now becoming more common of combining the intravenous with the intraspinal injection of the antimeningococcic serum. Time and experience alone will decide in how far this practice is superior to the intraspinal injection alone. The serum introduced into the subarachnoid space soon begins to escape into the blood; hence the necessity for its reintroduction if the meninges are to be kept bathed in the antiserum. The passage of the antiserum from the subarachnoid space into the blood leads to a general distribution throughout the organs. Possibly the higher dilution thus produced compared with the greater concentration secured from a direct injection of the larger volume of serum into the blood gives to the latter a special advantage in overcoming the so called meningococcemia.

It seemed desirable to determine, by direct agglutination tests, the agglutination titer of the blood and spinal fluid under the three sets of conditions; namely, after an intraspinal injection alone, after an intravenous injection alone, and after combined intraspinal and intravenous injection. This determination was made in monkeys; it could of course be carried out better with cases of epidemic meningitis under serum treatment. Unfortunately we were restricted by the scarcity of monkeys to one test of each condition. The result given in Text-fig. 1, *a*, *b*, and *c* must be regarded, therefore, as tentative only. Certain factors affecting the results are entirely ob-

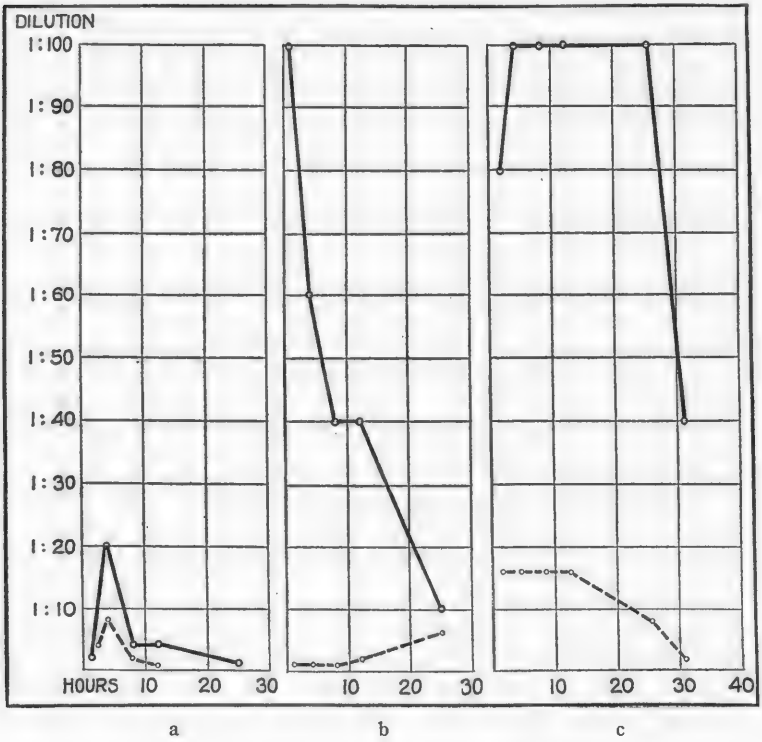
TABLE I.

Intraspinal injection.	Length of time after intravenous injection.	Dilutions of spinal fluid.					
		1:2	1:4	1:8	1:10	1:20	1:40
	hrs.						
	20	+	+	±	−	−	−
	25	+	+	±	−	−	−
Isotonic salt solution.	45	±	±	−	−	−	−
	69	±	−	−	−	−	−
	93	±	−	−	−	−	−
	20	+	+	+	±	−	−
	25	+	+	+	+	±	−
Normal horse serum.	45	++	++	++	+	+	+
	69	±	+	±	−	−	−
	93	+	+	±	−	−	−

scure; such, for example, as the delay after intravenous injection before the full expression of the agglutination titer declares itself. The three curves are, however, not only distinct, but suggest first that passage of the serum from the cerebrospinal fluid into the blood begins almost at once (Text-fig. 1, *a*), and second that the persistence of the titer in the blood and spinal fluid (Text-fig. 1, *c*) is sensibly affected by combined intraspinal and intravenous injection. However, we desire to repeat that not too much stress should be laid upon the results of these single tests.

Experiment 3.—Macacus rhesus G. May 14, 1918, 9.15 a.m. Injected intraspinally 3 cc. of polyvalent antimeningococcic serum. Blood was taken for

agglutination tests at 10.30 a.m., 1.15, 5.15, and 9.30 p.m. Lumbar puncture at 10.50 a.m., 1.25, 5.45, and 9.45 p.m. May 15. Bleedings at 10.45 a.m. and 4.18 p.m. Lumbar puncture at 10.50 a.m. and 4.23 p.m. The samples were tested May 16 for agglutinins with a parameningococcus, and the results are recorded in Text-fig. 1, *a*.



TEXT-FIG. 1, *a*, *b*, and *c*. (*a*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intraspinal injection of 3 cc. of antimeningococcic serum. (*b*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after intravenous injection of antimeningococcic serum and the intraspinal injection of normal horse serum. (*c*) Meningococcic agglutinins in the blood and spinal fluid of the monkey after combined intravenous and intraspinal injection of antimeningococcic serum.

Macacus rhesus H. May 13, 1918, 5 p.m. Injected intraspinally 2 cc. of normal horse serum. May 14, 8.10 a.m. Injected intravenously 20 cc. of polyvalent antimeningococcic serum. Specimens of spinal fluid and blood were taken at 9.10 a.m., 12.10 and 8.10 p.m., and at 9.10 a.m. on May 15. The results of the agglutination tests with parameningococcus on May 16 are recorded in Text-fig. 1, *b*.

Macacus rhesus I. May 14, 1918, 9.30 a.m. Injected intraspinally 3 cc. and intravenously 20 cc. of polyvalent antimeningococcic serum. Samples of blood and spinal fluid were removed at 11 a.m., 1.30, 6, and 9.40 p.m. May 15. Specimens taken at 10.50 a.m. and 4.25 p.m. The results of agglutination tests with parameningococcus on May 16 are shown in Text-fig. 1, *c*.

CONCLUSIONS.

Agglutinins for the meningococcus were not found in the spinal fluid of normal monkeys which had received antimeningococcic serum intravenously.

The intraspinal injection of isotonic salt solution, normal horse serum, or a culture of living meningococci allows agglutinins for the meningococcus to pass from the blood to the spinal fluid of the passively immunized monkey; and the rate of the passage is affected by the severity of the inflammation induced in the meninges.

The rates of elimination from the blood and spinal canal of meningococcic antibodies, as shown by the agglutination reaction, were compared in monkeys treated with immune serum (*a*) intraspinally, (*b*) intravenously, and (*c*) intraspinally and intravenously in combination.

(*a*) When immune serum is given intraspinally the agglutinins are very much diminished after 8 hours and practically disappear at 12 hours. They appear in the blood at the 4th hour after injection and quickly diminish.

(*b*) After intravenous injection of immune serum, when the meninges are inflamed, agglutinins appear in the spinal fluid in small amounts in about 12 hours and increase to the 25th hour. More than one-half of the agglutinins disappear from the blood within 8 hours and remain in low concentration at 25 hours.

(*c*) After combined intraspinal and intravenous injection the agglutinins remain in higher concentration in the spinal fluid and for a longer time than by method (*a*) or (*b*). The curve descends after 12 hours, and agglutinins are present at 25 hours. They remain in maximum concentration in the blood for 25 hours.

EXPERIMENTS ON THE MODE OF INFECTION IN EPIDEMIC MENINGITIS.

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Renewed interest has been aroused in the manner in which the meningococcus reaches the meninges from the upper respiratory mucous membrane. That the portal of entry of the meningococcus into the body is the mucosa of the nasopharynx is generally admitted. The point which has not been settled is whether the meningeal invasion is lymphatic or hematogenous. The most direct route would be along the lymphatics of the olfactory filaments to the meninges of the base of the brain; but no convincing evidence has been brought forward for that mode of invasion.

Westenhoeffer[1] conceived the idea that the meninges became infected from the sphenoidal sinuses; but later he abandoned that view.[2] Flexner[3] noted that when meningococci were injected intraspinally into monkeys by lumbar puncture film preparations of the nasal mucosa showed a small number of polymorphonuclear leucocytes believed to be derived from the inflamed meninges, containing Gram-negative diplococci resembling meningococci. He failed, however, to recover meningococci in cultures from the nasal mucosa of the inoculated animals, but he nevertheless discussed the possibility of a reverse process by which the meningococcus might be carried through lymphatics to the meninges in man.[4] This possibility was strengthened by the later observations on experimental poliomyelitis. The virus of poliomyelitis differs from the meningococcus in having a far wider range of pathogenicity for the monkey. While the meningococcus must be injected in considerable amounts directly into the subarachnoid space in monkeys in order to incite a fatal meningitis[3] and is entirely ineffective when

[1] Westenhoeffer, *Berl. klin. Woch.*, 1905, xlii, 737.

[2] Westenhoeffer, M., *Berl. klin. Woch.*, 1906, xliii, 1267, 1313.

[3] Flexner, S., *J. Exp. Med.*, 1907, ix, 142.

[4] The recovery of the organism was later accomplished in these laboratories from the nasal mucosa of monkeys which had received injections of the living meningococci intraspinally.

applied to the nasal mucosa, the application of small quantities of the virus of poliomyelitis to the latter is often followed by paralysis;[5] on the other hand, large doses of the virus often fail to induce infection when injected directly into the blood.[6]

Up to the present no satisfactory solution of the problem of the mode of infection in epidemic meningitis has been secured, and no considerable additions to our knowledge of the subject have been made since the pandemic of 1905–10. The appearance of the disease among the Western Armies has again stimulated interest in the problem. Naturally enough this interest has been primarily derived from clinical observations, and efforts at a better therapeutic control of cases. The newer observations indicate that the meningococcus is more frequently present in the circulating blood than has hitherto been supposed, and, especially, that a condition of hematogenous infection with the meningococcus may precede[7] any demonstrable infection or inflammation of the meninges, and, finally, that it is less rare than has been believed for a general infection with the meningococcus to exist independently of meningeal involvement.[8] The changing therapeutic point of view brought about by

[5] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, liv, 45.

[6] Clark, P. F., Fraser, F. R., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 223.

[7] Handa, H., and Nanjo, M., *Centr. Bakteriol., Ref.*, 1914, lxi, 82. Bonnel and Joltrain, E., *Bull. et mém. Soc. méd. Hôp. Paris*, 1916, xl, 75.

[8] Andrewes, F. W., *Lancet*, 1906, i, 1172. Liebermeister, G., *Münch. med. Woch.*, 1908, lv, 1978. Elser, W. J., and Huntoon, F. M., *J. Med. Research*, 1909, xx, 371. Bovaird, D., *Arch. Int. Med.*, 1909, iii, 267. Monziols and Loiseleur, *Bull. et mém. Soc. méd. Hôp. Paris*, 1910, xxix, 155. Chevrel, F., and Bourdinière, J., *Bull. et mém. Soc. méd. Hôp. Paris*, 1910, xxx, 165. Cecil, R. L., and Soper, W. B., *Arch. Int. Med.*, 1911, viii, 1. Barral, Coulomb, and Couton, *Bull. et mém. Soc. méd. Hôp. Paris*, 1912, xxxiii, 829. Bray, H. A., *Arch. Int. Med.*, 1915, xvi, 487. Sainton, P., and Maille, J., *Bull. et mém. Soc. méd. Hôp. Paris*, 1915, xxxix, 296, 374. Elliott, W. M., *Lancet*, 1916, ii, 1010. Pybus, F. C., *Lancet*, 1917, i, 803. Netter, A., Salanier, M., and Wolfrom, *Compt. rend. Soc. biol.*, 1916, lxxix, 973. Worster-Drought, C., and Kennedy, A. M., *Lancet*, 1917, ii, 711. Symmers, W. S., *Brit. Med. J.*, 1917, ii, 789. Anderson, W., McNee, J. W., Brown, H. R., Renshaw, A., McDonnell, J., Davidson, F. C., Gray, A. C. H., and Herringham, W. P., *J. Roy. Army Med. Corps*, 1917, xxix, 473. Sainton, P., *Paris méd.*, 1918, viii, 86. Zeissler, J., and Riedel, F., *Deutsch. med. Woch.*, 1917, xliii, 258. Thomsen, O., and Wulff, F., *Hospitalstid.*,

these experiences has culminated in the observations just published by Herrick[9] and others.[8]

The fact, now fairly established by studies carried out very early in cases of epidemic meningitis among our recruits, that a meningococcemia may precede an actual demonstrable meningococcus invasion of the subarachnoid space, has brought up the question whether the condition favored or hindered the development under these conditions of the meningitis itself. In civil practice, where cases are not observed so early in the attack, it is exceptional to have to deal with symptoms referable to irritation of the meninges but in which meningococci are present in the blood or even in the cerebrospinal fluid, without, however, being attended by cellular or chemical changes in the cerebrospinal fluid itself. In other words, under the latter circumstances the indications of inflammation are usually present in the meninges, calling for the intraspinal injection of serum. When, however, it happens that no evidence of inflammation is revealed by the lumbar puncture, the question has arisen as to the advisability of making any intraspinal injections of the serum whatsoever.

The reasons for doubt in this instance are two: first, whether any therapeutic benefit will follow; and second, and more important, whether the aseptic meningitis set up by the antimeningococcic serum may favor the local inflammatory attack of the meningococci. The latter point has been raised by Herrick because of the experiments of Flexner and Amoss[10] on the promoting action of an aseptic meningitis induced by serum and other agents on infection in poliomyelitis; but it disregards the important consideration that such an aseptic inflammation is only favoring when a non-immune serum is employed. An immune antipoliomyelitic serum equally sets up an aseptic inflammation, but it also prevents the infection's arising.[10,11] There is therefore no exact analogy to be found be-

1917, lx, 1192, abstracted in *J. Am. Med. Assn.*, 1918, lxx, 498. Baeslack, F. W., Bunce, A. H., Brunelle, G. C., Fleming, J. S., Klugh, G. F., McLean, E. H., and Salomon, A. V., *J. Am. Med. Assn.*, 1918, lxx, 684. Baron and Dumont, *Presse méd.*, 1917, xxv, 394.

[9] Herrick, W. W., *Arch. Int. Med.*, 1918, xxi, 541.

[10] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[11] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

tween these experiments on poliomyelitis and the hypothetic problem referred to in meningococcus infection in man.

On the other hand, the newer knowledge on meningococcus infection suggests that experiments be undertaken to determine whether an aseptic meningitis may not promote the passage of meningococci from the blood into the meninges and thus contribute to the inciting of meningococcus inflammation. The monkey is the most suitable animal for these experiments.

In the first place, the monkey can be given meningitis by an intraspinal injection of a suitable culture of the meningococcus. The infection in this case runs a mild or fatal course, depending on the virulence and dose of the culture employed. From the meninges meningococci escape into the general circulation but do not multiply and are quickly suppressed there. When the cultures are injected directly into the blood they do not appear in the meninges in normal monkeys but tend soon to disappear; a meningitis does not arise under these circumstances.

The preceding considerations led us to devise a series of experiments to determine the influence of an aseptic meningitis on the intravenous inoculation of cultures of virulent meningococcus in monkeys. The aseptic inflammation was incited by (*a*) normal horse serum, (*b*) normal salt solution, and (*c*) protargol. The last chemical not only induces an inflammation, but it paralyzes phagocytosis as well, and because of that action intensifies the local action of the meningococcus.[12]

EXPERIMENTAL.

The first experiment was arranged to determine the fact of the survival of the meningococcus in the blood and whether it would pass into the cerebrospinal fluid of a normal monkey. Meningococci vary greatly, as stated, in their initial power to set up a severe or fatal meningitis in monkeys on intraspinal injection.[3] When the effect is too slight the virulence of some cultures can be heightened by passage through monkeys or guinea pigs. All monkeys used were *Macacus rhesus*.

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1916, xxiii, 683.

Intraspinal Inoculation.—An irregular strain of the meningococcus, tending toward the para type, was passed through two monkeys by intraspinal injection, as indicated below, when it acquired a suitable degree of virulence for use. A sheep serum agar slant of a growth 14 hours old was suspended in 2 cc. of isotonic salt solution. Mar. 14, 1918, 4 p.m. Monkey 1 received the entire suspension intraspinally, by lumbar puncture. Mar. 15, 10 a.m. Animal ill. Lumbar puncture, 1 cc. of cloudy fluid containing polymorphonuclear cells and many meningococci. The cocci were mostly extracellular and of typical morphology. A blood culture taken at the same time was negative. Mar. 16, 11 a.m. Animal very ill. Lumbar puncture, 1 cc. of turbid fluid. Microscopic examination showed many pus cells and numerous Gram-negative diplococci, many phagocyted. Two drops of the fluid on sheep serum agar yielded abundant growth. The animal may or may not have recovered, but as the purpose was to recover the meningococcus for subsequent inoculation it was given 2 cc. of antimeningococcic serum intraspinally. Recovery followed.

The culture obtained on Mar. 15 from the spinal fluid was transferred on Mar. 17 to a tube of sheep serum dextrose agar and the growth suspended in the manner described and injected intraspinally at 11.45 on Mar. 18 into Monkey 2. 5.15 p.m. Animal ill. Temperature 39.2°C. Lumbar puncture, 1.5 cc. of turbid fluid under pressure. Microscopically there were large numbers of meningococci and pus cells; few cocci had been phagocyted. The culture yielded abundant growth. Blood culture positive. As the purpose again was to recover the culture, 2 cc. of antimeningococcic serum were injected. The animal gradually recovered.

A third suspension of this meningococcus was prepared in the manner described and injected intraspinally into Monkey 3 on Mar. 20 at 12.10 p.m. 6 hours later the monkey was very ill. Lumbar puncture was performed and antimeningococcic serum administered. Blood culture positive. 3 cc. of turbid fluid were recovered by the lumbar puncture. It contained large numbers of pus cells and meningococci, none intracellular. Mar. 21. Lively. Mar. 22. Completely recovered.

It is obvious from the brief descriptions given that the culture of meningococcus quickly rose in virulence and set up severe symptoms in shorter periods. The following experiments were made with this potent culture.

Experiment 1. Intravenous Inoculation.—Mar. 22, 1918, 11 a.m. *Macacus rhesus* given one-half of a suspension of 18 hour growth of culture of meningococcus obtained from Monkey 3 intravenously. 5 p.m. No symptoms. 5 cc. of blood withdrawn from vein. 3 cc. laked and plated. 2 cc. planted in dextrose broth. Former remained sterile; latter gave growth of meningococci.

Lumbar puncture yielded clear fluid devoid of cocci or cells. Mar. 24. No symptoms. Lumbar puncture, no fluid. No symptoms subsequently developed. Apr. 1. Animal lively. Apr. 2. Found dead.

Autopsy.—No visceral changes; no cause of death established. Microscopic examination of the central nervous system showed no lesions of any kind. The cause of death can only be conjectured. Possibly it was due to delayed endotoxin intoxication.

This experiment confirms many previously made which are to the effect that an intravenous injection of meningococci into monkeys followed by several lumbar punctures does not set up a meningitis even when the culture is active on meningeal inoculation.

In the next experiments the effects of aseptic meningitis were investigated. In these a strain of parameningococcus recently cultivated from the blood of a case of meningitis in man was employed. It was first tested by intraspinal injection into a *Macacus rhesus* and proved virulent.

Experiment 2. Intravenous Inoculation Following Intraspinal Horse Serum Meningitis.—Mar. 24, 1918, 4 p.m. *Macacus rhesus* received 2 cc. of normal horse serum intraspinally. Mar. 25, 10 a.m. Suspension of one-half of sheep serum dextrose agar slant culture in 10 cc. of isotonic salt solution injected intravenously. 5 p.m. 0.5 cc. of blood withdrawn from vein and placed in dextrose broth yielded a growth of meningococcus. Mar. 26, 10 a.m. Lumbar puncture, 0.5 cc. of slightly turbid fluid containing 400 polymorphonuclear cells per c.mm. No meningococci on stained films prepared from centrifuged sediment or in cultures. 5 p.m. same day. 5 cc. of blood taken, laked by addition of two parts of sterile distilled water, centrifuged, and residue smeared on plates. Innumerable colonies of meningococci appeared on the plates. 72 hours after the intravenous inoculation the animal was well; a blood culture was negative, and 0.5 cc. of clear fluid obtained by lumbar puncture was free of meningococci by microscopic and culture test. The monkey remained well.

This experiment was repeated with the same result. The interval between the intraspinal injections of horse serum and the intravenous inoculations of the culture was, however, 40 hours. 1 cc. of blood withdrawn 96 hours after the intravenous injection in dextrose broth yielded a pure culture of meningococcus.

Reference has already been made to the promoting action of an intraspinal injection of normal saline solution in poliomyelitis. As the salt solution sets up an aseptic meningitis of milder degree than

serum it was used in the experiments with meningococcus merely to exclude any protective local action of large numbers of polymorphonuclear leucocytes such as are brought into the meninges by horse serum.

Experiment 3. Intravenous Inoculation Following Intraspinal Saline Solution.—The culture of parameningococcus had been passed through two monkeys and recovered from the circulating blood. Apr. 23, 1918, 4 p.m. *Macacus rhesus* received 2 cc. of isotonic salt solution. Apr. 25, 10 a.m. (42 hours after intraspinal injection). Injected intravenously one-half of a 16 hour growth of meningococcus on a sheep serum dextrose agar slant in 10 cc. of isotonic saline solution. Apr. 26, 10 a.m. Lumbar puncture, 1 cc. of clear fluid obtained. Film preparations and culture from bottom of tube after long centrifugation revealed no meningococci. Blood culture of 5 cc. in blood dextrose broth yielded heavy growth of meningococci. Apr. 27, 10 a.m. 5 cc. of blood withdrawn for culture in dextrose broth yielded meningococci. Lumbar puncture, 2 cc. of slightly turbid fluid which was centrifuged at high speed. Film preparations showed polymorphonuclear leucocytes and few round cells but no meningococci. 1 cc. of the fluid containing the sediment planted in dextrose blood broth showed no growth. Apr. 29, 10 a.m. Monkey active and lively. Lumbar puncture, 0.5 cc. of slightly turbid fluid. No meningococci were found on microscopic examination and culture. Blood culture, 5 cc. in dextrose broth negative.

Protargol injected intraspinally not only sets up an inflammation of the meninges, but is antiphagocytic in its action against meningococci. Hence it was employed to promote an intraspinal infection by way of the blood stream.

Experiment 4. Intravenous Inoculation Following Intraspinal Protargol.—Mar. 21, 1918, 4 p.m. *Macacus rhesus* received intraspinally 2 cc. of a 0.5 per cent aqueous dilution of protargol. Mar. 22, 10.30 a.m. Injected intravenously one-half of a 16 hour slant culture on sheep serum dextrose agar of the virulent parameningococcus in 10 cc. of isotonic salt solution. 5 p.m. Withdrew 5 cc. of blood, which, after laking with sterile distilled water and centrifuging, were plated on blood agar and yielded a confluent growth of meningococcus. Mar. 23, 10 a.m. Blood culture, 5 cc. plated as before yielded about 200 colonies of meningococci. Lumbar puncture, withdrew 0.5 cc. of slightly turbid fluid. Microscopic examination of film preparation showed a few fragmented, poorly staining polymorphonuclear cells and debris but no meningococci. Culture in blood broth was negative. Mar. 24. Found dead.

Autopsy.—Some congestion of brain at the base. Cultures and film preparations from meninges, cortex, lateral ventricles, and base showed no meningococci or other microorganisms. Microscopic sections showed slight meningeal inflammation as indicated by a collection of leucocytes probably due to the protargol injection.

This experiment was repeated so that the observations extended over a somewhat longer period of time. Because of the early disappearance of the meningococci from the blood after injection several intravenous injections of living meningococci were made at intervals to prolong the period of the meningococcemia.

Experiment 5. Multiple Intravenous Inoculations Following Intraspinal Injection of Protargol.—Mar. 27, 1918, 4 p.m. *Macacus rhesus* injected intraspinally with 2 cc. of a 0.5 per cent aqueous dilution of protargol. Mar. 28, 11 a.m. Injected intravenously with one-half of a 16 hour growth on sheep serum agar of a virulent parameningococcus. 3 p.m. Blood culture positive; total leucocytes 10,200. Mar. 29, 11 a.m. Blood culture positive; lumbar puncture yielded 2 cc. of slightly turbid fluid containing fragmented cells but no meningococci. Cultures remained sterile. Mar. 30, 11 a.m. Lumbar puncture yielded 2 cc. of fluid; no meningococci found on culture or microscopic examination. Blood culture (5 cc.) positive; total leucocytes 7,600. Mar. 31, 11 a.m. Lumbar puncture, 0.5 cc. of fluid; no meningococci found by culture or microscopic examination. Blood culture (5 cc.) positive. Apr. 1, 11 a.m. Lumbar puncture, 3 cc. of fluid. No meningococci found by culture or microscopic examination. Blood culture (8 cc. in broth) negative.

The animal never showed any symptoms of meningitis and was allowed to rest 16 days to develop a partial immunity, for two purposes: first, to ascertain whether subsequent injections under conditions of partial immunity would allow meningeal invasion by the clumping of the organisms (*in vivo* agglutination) as sometimes happens in dogs injected with living pneumococci;[13] and second, to ascertain whether the organisms introduced by spinal injection would be found in the blood stream in the highly active immune animal.

Apr. 13. Injected intravenously one-half of a 20 hour growth of parameningococcus on slant serum agar in 10 cc. of isotonic salt solution. Apr. 20. Injected intravenously three-quarters of a 16 hour culture of parameningococcus. Apr. 27. Injected the entire culture. The animal remained well. Before injection serum agglutinated the homologous parameningococcus completely in a dilution of 1:1,200 and other parameningococci in 1:1,000. May 2, 5 p.m. Injected intraspinally in 5 cc. of isotonic salt solution entire 8 hour growth of virulent parameningococci on slant serum agar. May 3, 11 a.m. Total white blood cells 24,200. 5 cc. of blood withdrawn for culture yielded meningococci. Lumbar puncture, 0.1 cc. of slightly turbid fluid from which large numbers of meningococci were grown. May 4, 11 a.m. Growth obtained from 1.5 cc. of blood withdrawn for culture. Total white blood cells 40,400. Lumbar puncture, 2 cc. of turbid fluid from which cultures were positive. Microscopic exami-

[13] Bull, C. G., *J. Exp. Med.*, 1916, xxiv, 7.

nation showed numerous Gram-negative diplococci intracellular; few extracellular. May 5. Total white blood cells 12,400. Blood culture (5 cc.) negative. Lumbar puncture, 3 cc. of slightly turbid fluid containing many phagocyted Gram-negative diplococci, none extracellular. Cultures negative. May 6. Total white blood cells 12,000. Blood culture (5 cc.) negative. Lumbar puncture, 3 cc. of slightly turbid fluid. Culture negative. No organisms found on microscopic examination.

In this experiment the living virulent meningococci circulated in the blood for at least 72 hours during an aseptic inflammation of the meninges without passing through and causing meningitis. The monkey was actively immunized by intravenous injections so that the serum agglutinated the meningococcus in a dilution of 1:1,200. The intraspinal injection of meningococci was followed by meningococcemia lasting 48 hours, showing that whereas the passage of these organisms from the blood to the cerebrospinal fluid could not be brought about even under conditions promoting interchange, ready passage in the opposite direction from the meninges to the blood was accomplished when antibodies were circulating in the blood.

Experiments with Rabbits.

Austrian[14] reports that he was able to observe localization of the meningococcus in the meninges and typical fatal meningitis after intravenous injection in three rabbits out of twenty which had received a previous intraspinal injection of normal rabbit serum. In the rabbits which received no preparatory intraspinal injections localization did not occur.

However, it is stated in his typical protocol that after the intraspinal injection of normal rabbit serum "injury of the cord caused twitching of the tail and spastic palsy of the left hind leg, and dyspnœa, coma and ataxia developed." It is possible, therefore, that mechanical rather than chemical injury made possible the passage of the microorganisms from the blood to the spinal fluid. In order to test the validity of this view two series of rabbits were injected intraspinally with normal rabbit serum. In the first series great care was taken to avoid mechanical injury to the cord, while in the second series

[14] Austrian, C. R., *Bull. Johns Hopkins Hosp.*, 1918, xxix, 183.

the needle was introduced so that spastic paralysis followed the procedure. The animals of both series subsequently received intravenous injections of living meningococci.

Series I.—Five rabbits weighing about 2 kilos received intraspinally 1.5 cc. of normal rabbit serum. Half an hour later there were injected intravenously in 10 cc. of saline solution the growths from two 16 hour cultures on slant serum agar of a virulent irregular meningococcus. Rabbit 1 was etherized 4 hours later, No. 2 died in 6 hours, No. 3 was etherized at 8 hours, and Nos. 4 and 5 at 24 hours. Cultures and film preparations from the cord, cortex, pons, base of brain, and heart's blood showed no meningococci.

Series II.—Rabbit 6, weight 2.2 kilos, injected intraspinally with 1.5 cc. of normal rabbit serum on Oct. 15, 1918, 3 p.m. 3.05 p.m. Spastic; paralysis of both hind legs. Oct. 16, 10 a.m. No paralysis. Injected intravenously, in 10 cc. of isotonic salt solution, the 16 hour growth from two serum agar slants of a virulent irregular meningococcus. 4 p.m. No symptoms. Etherized and autopsied. Lumbar portion of cord hemorrhagic. Film preparations of cord and base of brain showed polymorphonuclear leucocytes and a few Gram-negative diplococci, none on cortex. Cultures from heart's blood and base of brain yielded irregular meningococci.

Rabbit 7, weight 2 kilos, received intraspinally 1.5 cc. of normal rabbit serum on Oct. 15, 1918, 3 p.m. 3.30 p.m. Spastic paralysis of left hind leg. Oct. 16, 10 a.m. Paralysis persisted. Injected intravenously in 10 cc. of isotonic salt solution 16 hour growth from two serum agar slants of a virulent irregular meningococcus. Oct. 17, 10 a.m. Paralysis persisted. No other symptoms. Etherized.

Autopsy.—Small hemorrhage in lumbar portion of cord. The blood vessels of the pia and dura were congested. Spinal fluid was turbid. Film preparations from spinal meninges, pons, and base of brain showed considerable number of polymorphonuclear leucocytes and many Gram-negative extracellular diplococci. Cultures from the spinal fluid, base of brain, and pons yielded meningococci, but the cultures from heart's blood were negative.

These experiments indicate that even under the conditions of congestion and chemical inflammation the meningococci do not pass from the blood to the spinal fluid in the rabbit unless a break in the continuity of the tissues occurs. The mechanical injury allows passage and localization of the meningococci. The results of the negative experiments in the monkey (Table I) correspond to those obtained in Series I with rabbits.

TABLE I.

Results of Intravenous Injections into Monkeys of Living Virulent Meningococci Following Intraspinal Injections of Substances Producing Chemical Inflammation.

Experiment No.	Date.	Previous intraspinal injection.	Intravenous injection.	Last positive blood culture obtained at.	Result.
	1918				
1	Mar. 22	None.	One-half 16 hr. growth on serum agar slant.	24th hr.	No localization in meninges.
2	" 25	2 cc. of normal horse serum.	" "	24th "	" "
3	Apr. 24	2 cc. of normal horse serum.	" "	96th "	" "
4	" 24	2 cc. of isotonic salt solution.	" "	48th "	" "
5	Mar. 22	2 cc. of 0.5 per cent aqueous suspension of protargol.	" "	24th "	" "
6	" 28	2 cc. of 0.5 per cent aqueous suspension of protargol.	" "	72nd "	" "

DISCUSSION.

In discussing the several experiments described in this paper it will be well first to formulate the problem which presented itself for solution and then the means at hand to be employed for the purpose.

There has been no convincing finding either in man or in experimental animals to show how the meningococcus reaches the meninges. Two possibilities are obvious: lymphatic and hematogenous extension. The first would carry the meningococci directly from the nasopharynx to the base of the brain; the other indirectly, by way of the general circulation, to the subarachnoid space.

As long as cases of frank meningitis only came under observation the meningococci were usually found with ease in the turbid cerebrospinal fluid and possibly also in the blood. Flexner[3] showed in his experiments on the intraspinal inoculation of meningococci in monkeys that passage of the organism takes place from the subarachnoid space into the blood. Hence the mere finding of the

meningococci in the circulating blood in cases of meningitis in man could not be taken to indicate one way or the other their relation to the meningeal infection.

In one of the experiments recorded here (Experiment 5) intraspinal injection of meningococci into the actively immune monkey was followed by the appearance of these organisms in the blood, where they persisted for 48 hours. The serum at this time agglutinated the particular strain of meningococci in a dilution of 1:1,200. This indicates that passage is far more easily accomplished by the meningococci in one direction, from the spinal fluid to the blood, than in the opposite direction, blood to spinal fluid.

The study of very early cases of infection in military establishments has thrown new light on the mechanism of meningococcus infection. For leaving aside the rare cases of meningococcemia without meningeal localization, it appeared that not infrequently cases would come under observation said to show no inflammatory changes in the meninges, as determined by a study of the spinal fluid, but with severe general symptoms of infection (including subcutaneous hemorrhages) in which meningococci were cultivable from the circulating blood. If these cases were not promptly treated and arrested by specific serum therapy, a meningitis is stated soon to develop. It may be that the presence of the meningococcus in the blood precedes their occurrence in the meninges or that the organism, on entering the cranial cavity and the blood simultaneously, is more readily recovered from the blood stream, or lastly that it is established in the upper portions of the central nervous system and overflows into the blood stream before evidences appear in the spinal fluid withdrawn at the remote and lower parts of the central nervous system.

In endeavoring to find a solution of the problem presented by experiments two points were kept in mind: first, that of a possible primary blood infection; and second, a promoting effect of the intraspinal injection of a serum on the localizations of the meningococcus in the meninges.

As regards the possibility of obtaining a decisive result by experiments on monkeys, the first point to be taken into account is the relative insusceptibility of the monkey to infection with the meningococcus. This degree of resistance is so great that Flexner[3] questions

whether, after an intraspinal injection, in which a fatal meningitis is set up, any actual multiplication of the microorganism takes place. He thinks it not impossible that from the beginning the injected micrococci are killed off, and the final fatal effect is caused by intoxication.

There can therefore be no very close analogy between the conditions experimentally induced and those existing in man when meningococcus infection arises. The experiments presented, however, are entirely clear on one point; namely, that in the monkey the meningococcus cannot be made to pass from the circulating blood, in which it is proved still to be surviving, to the aseptically inflamed meninges in such a way as to be detected there either by microscopic examination or in cultures. In other words, as far as the monkey is concerned the production of an aseptic meningitis is wholly without effect on the fate of meningococci injected into the blood stream.

The experiments are therefore in strong contrast to those made with the poliomyelitic virus, just as the great activity of the poliomyelitic virus when implanted on the nasal mucosa is in striking contrast to the innocuousness of the meningococcus applied to the same membrane. The experiments described in this paper emphasize, therefore, the unsuitability of the lower monkeys to solve the problem of the mode of meningococcus infections in man. And as far as they go they tend to show that an aseptic meningitis does not predispose the meninges to infection from the blood stream. Since in man the serum injected intraspinally is not normal but immune it can be presumed that the increased permeability of the meninges and choroid plexus induced by the chemical inflammation set up would be more than offset by the immunity principles introduced into the subarachnoid space along with the serum.

CONCLUSIONS.

The lower monkeys as represented by *Macacus rhesus* are resistant to a high degree to infection with cultures of the meningococcus introduced into the general blood.

The lower monkeys are less resistant to infection when the meningococcus cultures are injected directly into the subarachnoid space by lumbar puncture.

Relatively virulent cultures, which have been passed through several monkeys, acquire the power of surviving in the circulating blood of the monkeys for a maximum period of about 72 hours. Nothing has, however, been observed to indicate that the injected meningococci actually multiply in the blood.

It has not been found possible to direct the meningococci circulating in the blood into the cerebrospinal meninges of monkeys. In this effort an aseptic meningitis was induced by injecting horse serum, saline solution, or protargol into the subarachnoid space preceding the introduction of the meningococci into the blood.

In rabbits the meningococci were able to pass into the spinal fluid from the blood when a physical break in the continuity was made; however, under the conditions of chemical inflammation of the meninges the rabbit reacted just as the monkeys, and the organisms did not pass.

Because of the high insusceptibility of the monkey to infection with meningococcus, it is not believed that the experiments throw any new light on the mode of invasion of the body in man by that microorganism.

The experiments do not lend any support to the notion that an intraspinal injection of the antimeningococcus serum, early in the course of invasion of meningococcus in man, and possibly at a period at which the meninges do not yet show evidences of inflammation, favors its diversion from the blood stream into the subarachnoid space.

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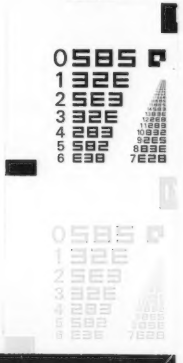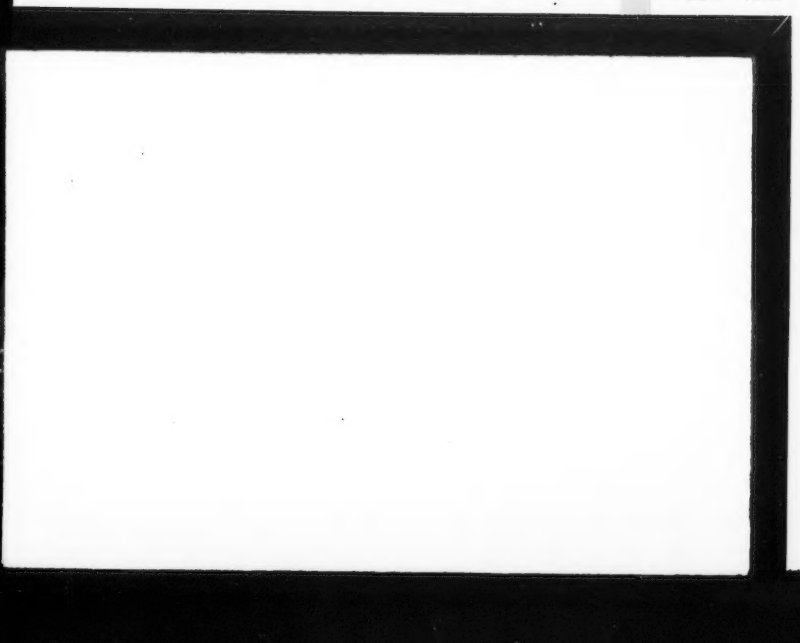
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